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Corso di Laurea Magistrale in Scienze e Tecnologie Animali

**Effect of feed restriction and energy source on digestive efficiency,
health status and growth performance of rabbits, and physiological
response to lipopolysaccharide (LPS)**

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ANNO ACCADEMICO

2012 – 13

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Abstract

The effect of feed restriction and dietary energy source (starch vs. fibre) on digestive parameters, growth performance and inflammatory response to lipopolysaccharide (LPS) stimulation were analysed in the rabbit after weaning, using 2x2 experimental design. The two experimental factors were feeding level (*ad libitum* –AL- vs. 75% of AL) and dietary energy source (starch –ST- vs. dietary fibre -DF). The restriction programme was applied by controlling the AL groups feed intake level for 3-4 days (ST100 and DF100) in order to readjust the theoretical amount of feed to give to other two 75% restricted groups (ST75 and DF75) from 35 to 64 days of age. Then all rabbits were re-fed *ad libitum* up to 71 days of age. The feed intake of two restricted groups at *ad libitum* re-feeding period increased and averaged 201.3 g/day/rabbit. During restriction, the live weight and the weight gain decreased significantly in the two restricted groups ($P < 0.001$). At re-feeding period restricted animals showed significantly lower weight gain (-8%) and feed conversion ratio (-10%) than AL ones. At 63 days of age the appendix weight of restricted-fed rabbits was significantly lower than AL ones (-7%) and during re-feeding, spleen weight was higher than AL ones (+6%; $P = 0.051$). The feeding level and the energy source did not affect the rabbits' health status. After 7 days of feed restriction the dry matter digestibility was increased by 2% ($P < 0.05$). The LPS injection (100 µg/kg body weight) led to a rise of body temperature but had not a significant effect on the response of animals in terms of fever in the four experimental groups. The main dietary energy source had no effect on the considered parameters.

Riassunto

La presente prova, condotta su conigli in post-svezzamento, è stata organizzata in un disegno sperimentale 2x2 e ha valutato gli effetti del livello alimentare e della fonte energetica (amido vs. fibra) sui parametri digestivi, prestazioni produttive e risposta infiammatoria dei conigli all'iniezione di lipopolisaccaride (LPS). I due fattori sperimentali erano livello alimentare (*ad libitum* -AL- vs. 75% di AL) e fonte energetica (amido -ST- vs. fibra -DF-). Il programma di razionamento è stato applicato controllando l'ingestione alimentare dei gruppi AL (ST100 e DF100) per 3-4 giorni al fine di riadattare la quantità di alimento, precedentemente calcolata a livello teorico, da somministrare ai due gruppi razionati al 75% (ST75 e DF75) dai 35 a 64 giorni di età. Successivamente, tutti i conigli sono stati alimentati *ad libitum* fino ai 71 giorni d'età. In seguito al periodo di restrizione alimentare e fino ai 71 giorni, l'ingestione è cresciuta nei gruppi razionati e si è attestata sul valore medio di 201.3 g/giorno/coniglio. Durante il periodo di restrizione alimentare, il peso vivo e l'accrescimento dei conigli appartenenti ai gruppi ST75 e DF75 sono diminuiti significativamente ($P < 0.001$). Una volta che questi ultimi sono stati alimentati nuovamente *ad libitum*, essi hanno evidenziato un minor accrescimento (-8%) e indice di conversione alimentare (-10%) rispetto ai conigli dei gruppi ST100 e DF100 ($P < 0.001$). A 63 giorni d'età, il peso dell'appendice dei conigli in restrizione alimentare era significativamente inferiore rispetto agli animali del gruppo AL (-7%) mentre, da 64 a 71 giorni d'età, il peso della milza dei conigli appartenenti ai gruppi ST75 e DF75 era tendenzialmente più elevato rispetto a quello dei conigli dei gruppi AL (+6%; $P = 0.051$). Il livello alimentare e la fonte energetica non hanno influenzato lo stato di salute dei conigli. Dopo 7 giorni di restrizione alimentare, la digeribilità della sostanza secca era aumentata del 2% ($P < 0.05$). L'iniezione di LPS (100 µg/kg di peso vivo) ha incrementato la temperatura corporea dei conigli, senza tuttavia generare delle differenze significative nei quattro gruppi sperimentali. La fonte energetica non ha avuto un effetto significativo sui parametri considerati.

Chapter 1

Introduction

1. Rabbit, meat production and meat quality

The rabbit meat production represents less than 1% (1.8 million tons) of the world meat production (FAO, 2009); the main meat productions are: pork (100 million tons), chicken (76 million tons) and beef (60 million tons). The consumption of rabbit meat in France was 1.2 kg/person/year in 2007 (Fortun-Lamothe and Gidenne, 2008).

Rabbit meat production is strongly developed in Mediterranean countries of the European Union, Italy is the first producer of rabbit following by France and Spain. Rabbit meat offers excellent nutritive and dietetic properties (review by Combes, 2004; Dalle Zotte, 2005; Combes and Dalle Zotte, 2005; Hernández and Gondret, 2006). Rabbit meat is rich in protein (20 ± 1.6 g/100g) with average lipid contents (8 ± 2.3 g/100g) and a good n-6:n-3 ratio (7.02 ± 3.62 mg/100g; Dalle Zotte and Szendrö, 2011) and it contains the lowest cholesterol levels (55 ± 18.5 mg/100g) respect of other meats (60, 74 and 81 mg/100g in beef, turkey and chicken, respectively; Dalle Zotte 2004). Along with high protein content, its contain also high levels of essential amino acids (EAAs). This high and balanced EEA and their easy digestibility give rabbit meat proteins a high biological value (Hernández and Dalle Zotte, 2010). Rabbit meat is rich in antioxidant and low in sodium compared to other types of meat (pork, chicken and beef) (Gigaud and Combes, 2007; Dalle Zotte and Szendrö, 2011).

In rabbit meat production the feed cost represents the largest part of all production costs reaching 60 to 70% of total costs (Maertens, 2009); feed efficiency as well as feeding strategy and health status, therefore have an important role in reducing the feed costs.

However, feeding strategies can modify growth and meat quality in rabbits. Feed restriction is a common practice in rabbit farms and it used to reduce post-weaning digestive disorders and improve feed conversion ratio (Hernández and Dalle Zotte, 2010). Restricted feeding strategy is widely practiced by French rabbit breeders (over 85%; Lebas, 2007), in this way we can improve feed efficiency and therefore reduce feed costs.

2. Digestive physiology system

Rabbits are monogastric herbivores and are hindgut fermentors because of their big caecum and colon and the absence of mammalian enzymes to break cellulose components (Cheeke, 1987; McNitt *et al.*, 1996); the resident bacterial ecosystem in the caecum allows the rabbit to digest a wide range of simple and complex carbohydrates (Figure1).

2.1. Stomach and small intestine

The stomach is the first important part of the digestive system in the rabbit, feed eaten by the rabbit quickly reaches the stomach. There it finds an acid environment. It remains in the stomach for a few hours (three to six), undergoing little chemical change. The stomach is always partially filled and it has a weak muscular layer. The pH is from 1 to 5 in respect to the region (*fundus* vs. *cardiacpyloric* region) (Gutiérrez *et al.*, 2002, 2003; Chamorro *et al.*, 2007; Orenge and Gidenne, 2007; Gomez-Conde *et al.*, 2009), the presence or absence of soft faeces (Griffiths and Davies, 1963), the time of feed intake (Alexander and Chowdhury, 1958) and the rabbit's age (Grobner, 1982). The contents of the stomach are gradually "injected" into the small intestine in short bursts, by strong stomach contractions.

As the contents enter the small intestine they are diluted by the flow of bile, the first intestinal secretions and finally the pancreatic juice. After enzymatic action from these last two secretions the elements that can easily be broken down are freed and pass through the intestinal wall to be carried by the blood to the cells. The main part of the digestion and the absorption occurs in the small intestine through the mucosa. Most of amino acids and starch of the total feed intake are digested at the end of ileum (Gutiérrez *et al.*, 2002; Garcia *et al.*, 2005; Carabaño *et al.*, 2009). The particles that are not broken down after a total stay of about one and a half hours in the small intestine enter the caecum. There they have to stay for a certain time, from two to 12 hours, while they are attacked by bacterial enzymes. Elements which can be broken down by this new attack (mainly volatile fatty acids) are freed and in turn pass through the wall of the digestive tract and into the bloodstream.

2.2. Large intestine and caecum

The rabbit caecum is very large and has a spiral form; it constitutes 40% of the total capacity of the gastrointestinal tract (Jenkins, 1999). Because of the specialized musculature of the large intestine, the rabbits are able to excrete hard faeces from slowly fermentable fibres in the colon. With a series of involuntary muscular contractions they move the fibrous particles through the colon to the anus and liquid and small particles to the caecum for further fermentation (Cheeke, 1987; Carabaño and Piquer, 1998; Leng, 2008). Hard faeces are produced after 4 hours of feed intake. Soft faeces (or caecotropes) are produced after 8 hours of feed intake from nonfibrous components in the rabbit's caecum. Caecotropes contain more nutrient components (protein, crude fibre, K and nicotinic acid) respect of hard faeces (Carabaño and Piquer, 2003). Volatile fatty acids (VFAs) and microbial cells are the principal products of soft faeces; the absorption of VFAs happens in the caecum. The rabbits consume their feed at night and caecotrophy (the consumption of caecotropes) takes place during the day (Carabaño and Piquer 1998; Lebas *et al.*, 1997).

Caecotrophy regulation depends on the integrity of the digestive flora and is governed by intake rate. Experiments have shown that caecotrophy starts eight to 12 hours after the feeding of rationed animals, or after the intake peak of animals fed ad libitum. In the latter case, the intake rate and hence the function of caecotrophy are governed by the light regime to which the animals are subjected.

Caecotrophy also depends on internal regulatory processes as yet not understood. In particular, the removal of the adrenals halts caecotrophy. Cortisone injections of animals without adrenals causes the resumption of normal behaviour. The digestive process of the rabbit appears to be highly dependent on adrenalin secretions. Hypersecretion associated with stress slows down digestive activity and entails a high risk of digestive ailments (Lebas *et al.*, 1997). Implementing rabbits with a collar preventing caecotrophy led to significant reduction of digestion and growth rate by 50% (Irlberck, 2001; Leng, 2008).

2.3. Feeding behaviour

From the third week of life the young rabbits begin to move about, taking a few grams of mother's milk and a little drinking water if available. In a few days the intake of solid feed and water will exceed the milk intake. During this period the changes in feeding behaviour are remarkable: the young rabbit goes from a single milk feed a day to a large number of alternating solid and liquid feeds distributed irregularly throughout the day: 25 to 30 solid or liquid meals every 24 hours. The number of solid meals, stable up to 12 weeks, tends to decrease slightly thereafter. The total feeding time in a 24-hour period exceeds three hours at age six weeks. It then drops off rapidly, to less than two hours. At any age, feed containing over 70 percent water, such as green forage, will provide rabbits with ample water at temperatures below 20°C. Much more liquid and solid feed is consumed in the dark than in the light. Intake in experimental hutches is very high just before the lights are switched off. As the rabbit grows older the nocturnal nature of its feeding habits becomes more pronounced. The number of feeds during light periods drops and the morning "feeding rest" tends to lengthen. The feeding habits of wild rabbits are even more nocturnal than those of domesticated rabbits (Lebas *et al.*, 1997).

3. Immune system

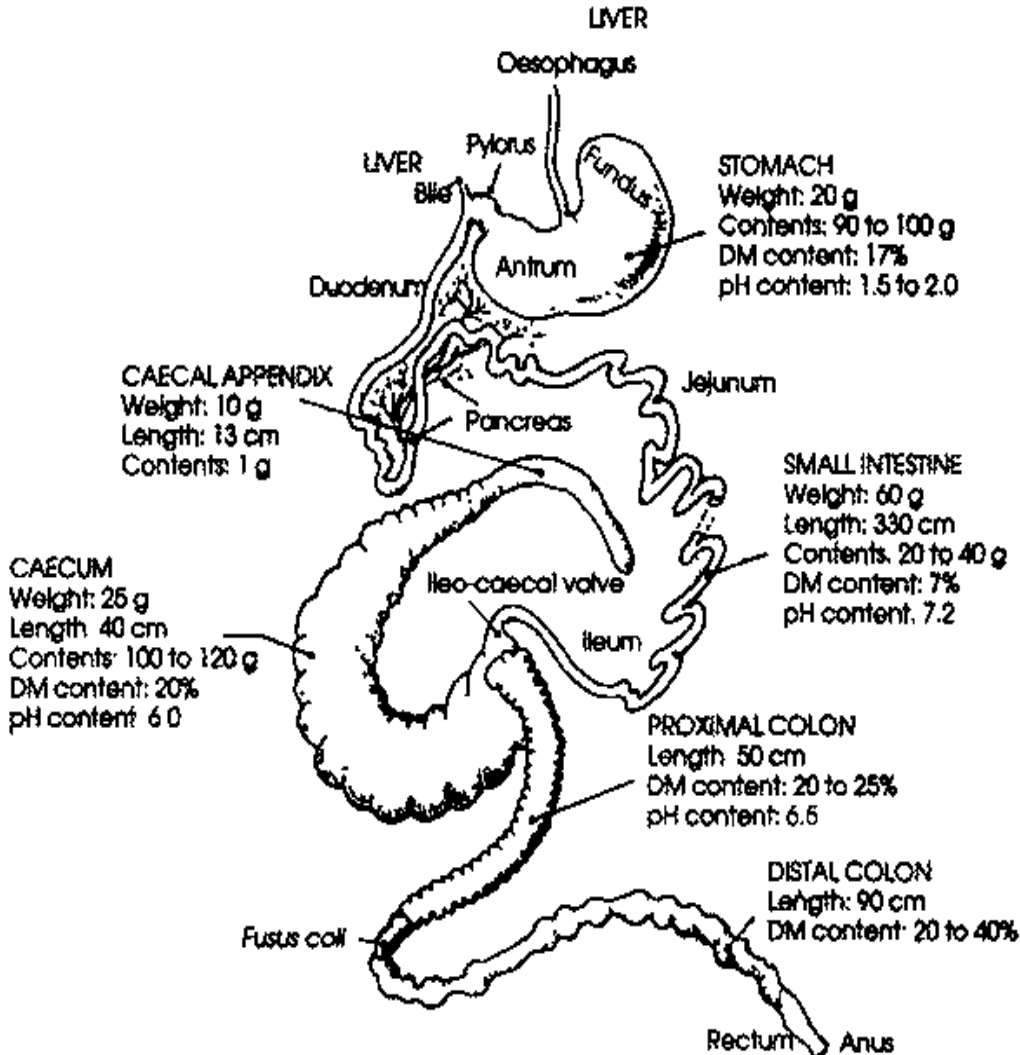
The development of the immune system in rabbits happens in three periods. First period occurs in foetal and neonatal rabbits and depends on genetic factors and placental transfer during gestation (Carabaño *et al.*, 2008).

The second period is the creation of a primary antibody repertory, the host's interaction with the intestinal microbiota is the cause of the generation and development of the primary

antibody repertory in the GALT (gut associated lymphoid tissue) between 4 and 8 weeks of age. (Lanning *et al.*, 2004; Mage *et al.*, 2008).

The last period is the formation of a secondary antibody repertory with an increase in the number of B cells in the secondary lymph organs (lymph nodes, spleen and GALT) in adult rabbits (Lanning *et al.*, 2004; Carabaño *et al.*, 2008).

Figure1. rabbit diegestive physiology system (Lebas *et al.*, 1997)



Note: Numerical values are those observed in the New Zealand White breed, aged 12 weeks, fed a complete balanced pelleted feed.

3.1. The Gut Associated Lymphoid Tissue (GALT)

The GALT's role is the identification of foreign antigens ingested through the feed consumption. The lymphoid tissues of the intestine of the rabbits are divided in two forms:

1. Organized form: in lymphoid follicles allotted in Peyer's patches (PP), the *vermiform appendix* and *sacculus rotundus*. A part of the cells of the intestinal immune system are made in these follicles and the immune response also begins there.
2. Diffused form: in the lamina propria (LP) and between the epithelial cells of the intestinal mucosa. It is disseminated along the gut. More immunoglobulins (Ig) are produced in LP (Carabaño *et al.*, 2008).

The *sacculus rotundus* located at ileo-caecal junction and the *vermiform appendix* located at the caudal end of the caecum; these two structures contain several hundred dome-follicles organized in adult animals quite similarly to PP; Rabbits have between 2 to 10 PP along the small intestine with numerous dome-follicles (Mage, 1998). *Sacculus rotundus*, *vermiform appendix* and PP uptake macromolecules and micro-organisms from the lumen to the lymphoid cells (Simecka, 1998). Peyer's patches and the appendix reach their maximum size at 6 weeks of age in rabbits (Weinstein *et al.*, 1994) in healthy rabbits, the appendix lymphoid changes take place during the first 12 weeks of age with changes in shape and decrease in the number of follicles (Dasso *et al.*, 2000). After their differentiation and maturation in primary lymphoid organs, the lymphoid cells migrate to secondary lymphoid organs like the spleen, the lymph nodes and the GALT (Fortun-Lamothe and Boullier, 2007). Rabbits, like other mammals, have two types of immune responses: the innate and the adaptive immune response; the innate immune response is non-specific and constitutes the first line against pathogens. The innate immune response is itself divided into the cellular response and the humoral response that embodies the inflammatory response. The adaptive immunity is antigen specific foreign elements in the gut and is activated after the innate response.

3.2. Induction of the inflammatory response

LPS (lipopolysaccharide, an essential component of the cell wall of Gram- bacteria; MacDonald *et al.*, 2011) has been used in particular to induce inflammation in rabbits (Lee *et al.*, 1998; Huang *et al.*, 2008). LPS stimulated the secretion of TNF (Tumor Necrosis Factor) and other proinflammatory cytokines (Dinarello, 1992; Mathison *et al.*, 1988). An intravenously injection of LPS (50 and 100 µg/kg of BW) in rabbits (weighing 2.5 to 3.0 kg) caused fever and inflammation (Brito *et al.*, 1995). When 25 µg of LPS were injected intraperitoneally in restricted fed mice (60% of *ad libitum* group from 8 to 12 week of age),

the TNF- α at the peak point was significantly lower in restricted group respect of AL ($P<0.05$); the injection of LPS induced a proinflammatory response in mice (Matsuzaki *et al.*, 2001). Two diets differing in level of energy (80% and 75% of the level of energy in control group) in mice infected by a parasite *H. polygyrus* led to a decrease in expression of IFN- γ (interferon), IL-4 and IL-5 and their effectors responses (IgE, IgG1 and eosinophils) in gut or splenic lymphoid tissues (Koski *et al.*, 1999).

4. Digestive pathology

This part is focusing on the specific digestive disorders that cause high mortality and morbidity in rabbits.

Diarrhoea is a serious threat in young rabbits (from 4 to 10 weeks of age) and it is rare in adult rabbits. The rabbit's reaction to disease takes a form of intestinal disturbance and appears frequently as diarrhoea. Before diarrhoea first symptoms, the feed intake is generally sharply reduced, and rate of passage is longer. Caecotrophy is also affected with the slowing peristalsis, and the reduction of feed passage through the intestine could lead to stop the caecotrophy. The intake reduction or the slow feed passage is associated to a lower fermentative activity, and thus the caecal pH increases, and the intestinal microbiota is modified (*Escherichia coli* becomes dominant for instance when an enteropathogenic *E. coli* is present).

Feeding is one the most important preventive factor to reduce the risk of occurrence of diarrhoea. Feed composition, like low fibre levels (or high energy), too high level of protein, and sudden feed changes may increase the risk of diarrhoea. On the other hand, also chemical agents, such as some antibiotics and high nitrate content in water, viruses, bacteria and intestinal parasites are specific causes of diarrhoea. The most important parasite causing diarrhoea in rabbits is *coccidia*. It is a protozoan and belongs to the genus *Eimeria*. *Eimeria* develop in the epithelial cells of the intestine and the liver of rabbits. *Coccidia* are the specific pathogenic agents for *coccidiosis* in rabbits; out of diarrhoea, other clinical signs are weight loss, low feed and water intake, disease-spreading and eventually death. Diarrhoea appears between 4 to 6 days after contamination (depending on *coccidia* species) and it is the first visible sign of infection.

Bacterial enteritis is another cause of diarrhoea in rabbits and they are classified in two groups:

1. Mucoïd enteritis: it is a special form of diarrhoea in growing rabbits, the colon and rectum are filled with a considerable amount of mucus.

2. Enterotoxaemia, colibacillosis, typhlitis: they are different names referring to the types of enteritis and they are very similar; death can occur before diarrhoea appears. Under autopsy the caecum is swollen and blotchy with red striations (Lebas *et al.*, 1997).

In France the mortality rate due to enteritis in rabbits is around 11-12% (Koehl, 1997), whereas with the use of antibiotics it is currently around 8.5% (Lebas, 2008). Digestible disorders cause growth depression and bad feed conversion and are the most important cause of morbidity in rabbits.

Epizootic Rabbit Enteropathy (ERE) was a great cause of mortality and morbidity rate (up to 70%) in growing rabbits in the last 10 years in Europe. Feeding strategies has been proposed for growing rabbits, such increasing the fibre level or restricting the feed intake, as a solution to control ERE or other digestive disorders.

5. Nutritional need

Rabbits are also called *concentrate selectors* because they select the more tender, succulent and most nutrient-dense plants in natural settings. This is a practice that allows them to meet the dietary requirements for their high metabolic rate (Cheeke, 1994). Rabbits have 65 to 80 g/kg BW feed intake and a fast feed transit time (19 h) (Carabaño and Piquer, 1998). They need to consume 5% of their BW with an energy dense diet and 8% of their BW with forage alone diet (Leng, 2008). Insufficient levels of fibre and/or too much starch often leads to diarrhoea (Irlberck, 2001). Compared with polygastrics the rabbit has a low fibre digestion capacity (14% for alfalfa hay in rabbits, 44% in cattle and 41% in horses) (McNitt *et al.*, 1996). A correct feed for rabbits contains high levels of fibre (around 20% ADF) to maintain a good microbial activity in the gut (Gidenne *et al.*, 2010).

5.1. Dietary fibre

Defining the dietary fibre and estimating its concentration in different feeds is important to limit and reduce digestive problems, diarrhoea and mortality (Gidenne, 1997; Gidenne *et al.*, 2000; Bennegadi *et al.*, 2001; Gidenne *et al.*, 2010). The plant cell walls are composed of several polysaccharides (cellulose, hemicelluloses, etc.) and polyphenols (lignin). According to the plant age, the cellulose is arranged in microfibrils that are implanted in a matrix of lignin (phenylpropane units) which fastens other matrix polysaccharides (and some glycoproteins) such as hemicelluloses (arabinoxylans, xyloglucans...) and pectins. According to the structures and properties of cell wall polymers five classes of fibre were defined: lignin, cellulose, hemicelluloses, pectic substances (4 water insoluble classes) and one class

of water soluble non starch polysaccharides and oligosaccharides, fructans, resistant starch and mannans (Gidenne *et al.*, 2010).

Lignin tends to make the cell wall harder and more resistant to various bacterial enzymes. Young grass contains 5% of lignin reaching 12% at maturity.

Cellulose could be used like backbone of the plants and it is the most structured polysaccharide of the cell wall. Legums contain 40-50% of cellulose, forage and beet pulps contain 10-30% per kg of dry matter.

Hemicelluloses can make strong hydrogen bonds with cellulose. They are a group of several polysaccharides; xylose is their backbone. Forages contain 10-25% of hemicelluloses per kg of dry matter whereas grains and roots contain about 2-12% of hemicelluloses. The sources of hemicelluloses in rabbit feed are cereals, beet pulp and also soybean cell walls.

Pectins are composed of polygalacturonic acid. Leguminous plants and fruits contain high levels of pectins; sugar beet pulps contain 25% of pectins per kg of dry matter whereas leguminous plants contain 5-10% of pectin.

Water soluble polysaccharides and oligosaccharides are in low proportions in rabbits feed, 2-4% of dry matter in wheat and barley, 5-8% of dry matter in lupin, pea and soybean seeds (Gidenne *et al.*, 2010).

5.2. The dietary energy source in diet (Starch vs. Fibre)

Starch is the main dietary energy source in rabbit feed, and its role is increasing the diet's energy concentration and improving feed conversion ratio (FCR) (Hernández and Dalle Zotte, 2010). The main part of the digestion of the starch takes place in the small intestine and the most important enzyme is the pancreatic amylase. Starch undigested in the small intestine is quickly hydrolyzed and fermented to produce VFAs and lactate in caeco colic segment (Blas and Gidenne, 2010).

Xiccato *et al.* (2008), showed sanitary risk was reduced by two thirds, increasing digestible fibre/starch ratio from 1.0 to 2.5 in two different diets associated with early antibiotic treatment.

Several large scale experiments (Gidenne *et al.*, 1998b; Gidenne *et al.*, 1999; Pinherio and Gidenne, 1999; Bennegadi, 2002) were performed with the aim of improving the fibre requirement of the growing rabbit; it was demonstrated that a reduction of dietary ADF (Acid Detergent Fibre) from 19 to 15%, increased sanitary risk (mortality + morbidity) from

18 to 28%. On the contrary, a dietary increase of ADL (Acid Detergent Lignin) from 1 to 5% reduced the sanitary risk from 70 to 35%.

Fibre is the most important factor for fermentative activity in rabbits but replacement of starch by fibre and fat in the diet didn't change significantly sanitary status in does (Lebas and Fortun-Lamothe, 1996; Pascual *et al.*, 1998, 1999; Quevedo *et al.*, 2006). A good balance between digestible fibre (pectins and hemicelluloses) and less digestible fibre (cellulose and lignins) is necessary to reduce the risk of diarrhea after weaning (Gutiérrez *et al.*, 2002; Gomez-Conde *et al.*, 2004; Soler *et al.*, 2004; Gidenne *et al.*, 2004; Gidenne and Licois, 2005; Fabre *et al.*, 2006)

Replacing starch by digestible fibre with a constant level of lignocelluloses (ADF = 18%) reduced slightly the feed consumption (-3.6%) while the growth rate remained unaffected, mortality caused by acute diarrhoea was reduced from 6.7% to 2.4% (Perez *et al.*, 2000). High starch diets could be less digested in the young rabbits, since the maturation of enzymatic system is not completed before 42 days of age (Scapinello *et al.*, 1999). It is worthwhile to limit the starch level in diets to 14% during the first part of the post-weaning period (prior to 42 days of age) to improve the digestive efficiency (Perez *et al.*, 2000). On the other hand, a level of 18% of starch in the diet during the finishing period has no major impact on digestive security in rabbits (Perez *et al.*, 2000).

The digestible energy (DE) concentration of a feed is related to the growth rate, and to the feed conversion rate (intake/growth) Theoretically an increase of fibre reduces the energy digestibility (Dehalle *et al.*, 1981; Partridge *et al.*, 1989) due to the incomplete digestion of fibres, and to energy losses in the caecum (production of gases and heat of fermentation) (Gidenne and Perez, 2000). However, replacing digestible fibre with starch did not greatly change the energy and protein retention in rabbits; reversely, reducing the ratio of ADF/starch from 1.5 to 0.75 did not influence significantly the digestive efficiency for fibre (but reduced that of energy), whereas the transit time in caeco-colic segment was longer (12 to 25 hours) (Gidenne and Perez, 2000).

Gruesco *et al.* (2013), with different diets substituting starch with soluble fibre (high starch 159 g/kg, low soluble fibre 54g/kg and low starch 108 g/kg, high soluble fibre 97 g/kg) with the same level of ADF, observed that mortality during fattening was reduced from 26.3 to 16.7% with the high soluble fibre diet. In this study soluble fibre insinuated an increase in digestibility of each of the cell wall components that led to a significant decrease in pH of soft faeces content (from 5.9 to 5.75).

6. Restricted feeding

Among the environmental factors that influence the interaction between microbiota and mucosa in the digestive system of rabbits, diet has an important role. Feed intake level was studied mainly for improving the meat quality (Parigi Bini *et al.*, 1994; Dalle Zotte *et al.*, 2001; Dalle Zotte *et al.*, 2005a; Dalle Zotte *et al.*, 2005b; Matics *et al.*, 2008) and energy metabolism in animals (Dalle Zotte *et al.*, 2003a; Dalle Zotte *et al.*, 2003b). More recently, post-weaning restriction strategies are widely used by rabbit breeders in France to reduce the risk of digestive troubles (Gidenne *et al.*, 2012).

Feed restriction can be applied in two ways: by diminishing the access time to feed or decreasing the quantity of feed distributed (Feugier, 2002; Szendrő *et al.*, 2000; Dalle Zotte *et al.*, 2005). Recent studies showed that reducing the quantity of feed was more precise to ensure the level of feed restriction (Gidenne *et al.*, 2011). Feed restriction strategies reduced the feed costs from 2 to 10% (Duperray and Guyonvarch, 2009). However a reduction of 20% of feed intake during 20 days (from 38 to 68 days of age) led to a slightly lower slaughter weight (-3.8%) (Gidenne *et al.*, 2008).

A 25% reduction of the intake level led to a better digestive efficiency of energy and protein but a scarce improvement in lipid or fibre digestion in growing rabbits (Xiccato *et al.*, 1992) or in adult rabbits (Lebas, 1979; Xiccato and Cinetto, 1988; Fodor *et al.*, 2001).

Gidenne and Feugier (2009) reported that restricted feeding (60% of *ad libitum*) led to a high retention time (65%) of digesta particles in the caeco-colic segment, and sharply modified the caecotrophy rhythm in the growing rabbits.

In a large scale experiment managed by the GEC (Group Experimentation Cunicole) in six experimental sites, the effect of feed restriction (60%, 70% and 80% of *ad libitum* intake for 21 days after weaning and then *ad libitum* to slaughter) on growth performance and health status of growing rabbits was studied. The mortality was significantly reduced (-9%) in all restricted fed rabbits and the morbidity was also reduced (-6%) for two groups (60%, 70% of *ad libitum*). On all sites diarrhoea or caecal impaction was the cause of mortality and morbidity (Gidenne *et al.*, 2009). Faecal apparent digestibility during the first week of application of the restriction strategy was not improved, since rabbits need a minimum of 8-10 days to adapt to the restricted feeding (Gidenne and Feugier, 2009).

During the period of restriction, growth was reduced by 20% (in rabbits restricted by 80% of *ad libitum*); a linear reduction between feed intake and growth in rabbits fed with 80% of the *ad libitum* was observed (Gidenne *et al.*, 2009). Feed conversion rate during the restricted period was reduced from 5% to 10% or attained a similar value to that observed in the *ad libitum* group, with a large variation according to the studies; after two weeks of re-

feeding the slaughter weight remained 5 to 10% lower than for the *ad libitum* rabbits (Gidenne *et al.*, 2011).

Similarly, a strategy of feed restriction was studied in pigs, during 3 to 8 days post-weaning (50% of *ad libitum* on d3, a minimum ration 100 g/pig/day on d4, d5 and 6, 200 g/pig/day on d7 and 8, and then *ad libitum*): a reduced post-weaning diarrhoea frequency was observed, but it was not significant in the periods before and after of feed restriction. The body temperature during the feed restriction was slightly but significantly lower (-0.2 °C) in the restricted group. This might be due to a lower energy level in restricted group to maintain the body temperature or more infections in control group (Ranzer *et al.*, 1996).

Also in poultry, restricted feeding (85% of *ad libitum* group, from 4 to 14 days of age and diminished by daily step to 5% at 21 days of age then slaughter at 37 days of age) reduced the mortality rate due to leg problems and ascites (0.9 vs. 1.9% for ascites and 0.0 vs. 4.6% for leg problems) (Wijttten *et al.*, 2010).

During the restriction period the rabbit digestive tract development (organ weight) was reduced (Schloutaut *et al.*, 1978; Perrier and Ouhayoun, 1996) and after re-feeding freely, the weight of the full digestive tract (organ + digesta) was higher (10%) than that of the *ad libitum* group (Gidenne *et al.*, 2011).

In order to minimize the negative effects of feed restriction on growth and slaughter yield it is relevant to optimize the feeding strategies by modulating the nutritional quality of the feed. In 2012 a study was conducted by the GEC group in order to compensate for the reduced growth of the feed restricted rabbits without negatively impacting the sanitary parameters. The possibility of increasing the dietary energy level of the feed was studied. Restricted feeding reduced by 7% the growth during the overall experimental period. If a high energy diet is fed the reduction of growth is only of 5%. The use of a high energy diet did not impact the sanitary parameters while the beneficial effects of feed restriction were confirmed: the morbidity rate was lowered from 12% to 9% and the mortality was lowered from 11% to 8.5% (Knudsen *et al.*, submitted).

Objectives of experiment

This study aimed to analyze the effect of short-term post-weaning feed restriction (75% of *ad libitum* level) and the effect of the main dietary energy source (starch vs. fibre) on several digestive parameters, growth performance and inflammatory response in rabbits.

It also aimed to confirm the beneficial effects of a restricted feeding at 75% of the voluntary feed intake with two feeds containing the same levels of digestible energy (2415 kcal/kg). Furthermore, the research investigated on the effect of dietary structural (fibres) and non-structural (starch) carbohydrates on rabbits' growth and health, and tried to deepen the knowledge on the mechanisms implicated in the positive response of the rabbit to feed restriction in terms of health and feed efficiency.

The variables measured included the sanitary status, the growth performances, the digestive efficiency, the weight of appendix and spleen and the inflammatory response to LPS stimulation.

The beneficial effects of feed restriction on health, feed efficiency and nutrient digestion and also the beneficial effects of a digestible fibre enriched diet on growth and immune system are the hypothesis of this experiment.

Chapter 2

Materials and Methods

This protocol belongs to a wide study (RAMIFI) conducted in collaboration with the GEC group (Group Experimentation Cunicole).

1. Experimental design

The study was divided in two experiments: a pre-experiment and a main experiment. The pre-experiment aimed to define the adequate amount of an inflammatory agent (Lipopolysaccharide, LPS) to inject to the rabbits in order to obtain a measurable inflammatory response. Four doses of LPS were tested (50, 75, 100 and 150 µg/kg of BW). We used 40 rabbits aged 42-43 days, it takes just 2 days and it was conducted one month before the main experiment. The first day the dose 100 µg/BW of LPS and T₁ (first control group) and the second day doses 50, 75 and 150 µg/BW of LPS and T₂ (second control group) were administered.

The main experiment was conducted simultaneously in a 2x2 experimental design. The two factors analyzed were: the feeding level (*ad libitum* vs. restricted at 75% of the *ad libitum* intake) and the main dietary energy source (starch vs. Dietary Fibres).

The groups of animals fed *ad libitum* were named: DF100 (Dietary Fiber *ad libitum*) and ST100 (Starch *ad libitum*)

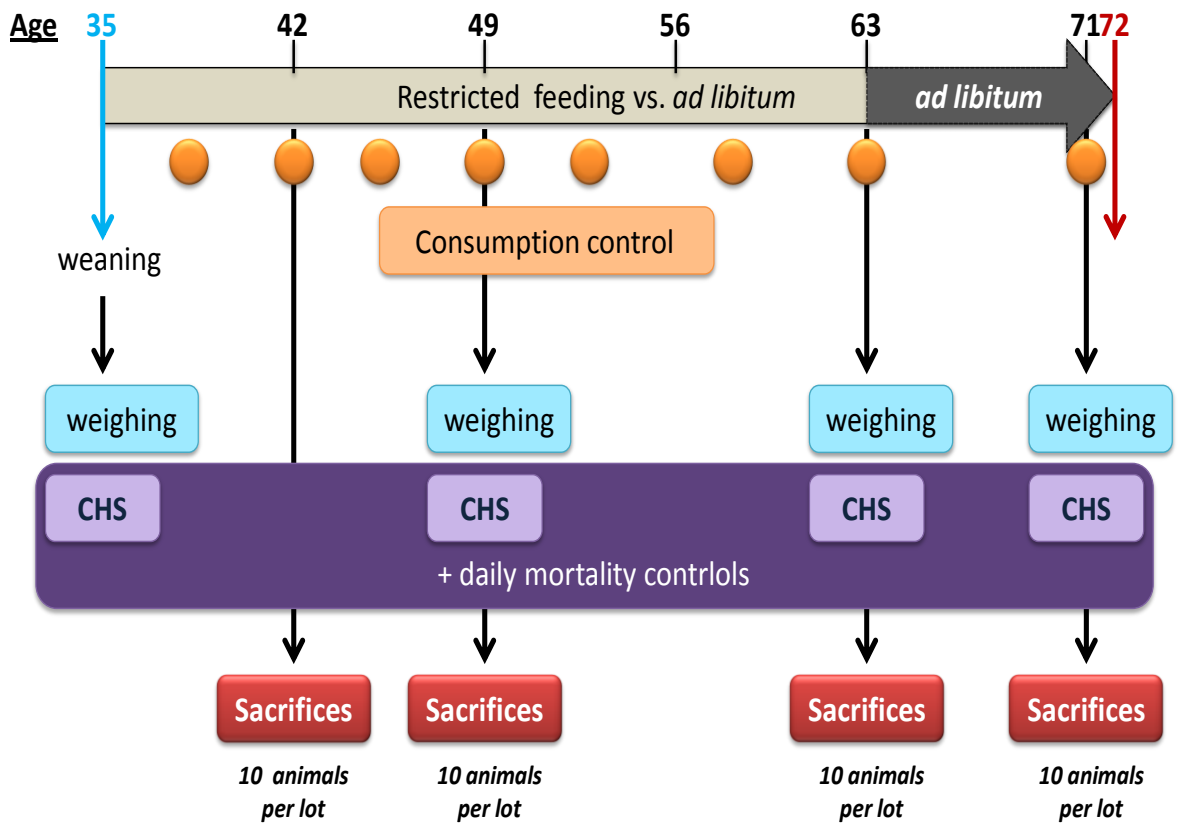
The groups of animals feed restricted at 75% of the *ad libitum* intake were named: DF75 (Dietary Fibre restricted at 75%) and ST75 (Starch restricted at 75%)

In the main experiment the animals at 35 days of age were randomly assigned to the different groups. The main experiment was divided in two parts, one considered 320 rabbits (big part) housed in collective cages to evaluate the live performances and the health status, whereas the other one used 48 rabbits (small part) housed in individual cages, to perform the digestibility experiment. All animals were divided into 4 groups according to the four experimental treatments (DF100, ST100, DF75, ST75) from 35 to 64 days of age. Restricted groups were re-fed one week before the end of the experiment (from 64 to 71 days of age).

2. Housing, animals and the environmental conditions

INRA hybrid rabbits were used and the does were inseminated in heterospermia. Environmental conditions in the rearing farm complied with standards in animal experimentation, including the ambient temperature and ventilation. The light was turned on for a period of 10 h per day between 1 a.m. and 11 a.m.

experimental design



CHS: Control of health status⁷

3. Pre-experiment

For this experiment we used 40 INRA hybrid rabbits of 35 days of age housed in collective cages (6 cages with 6 rabbits and one cage with 4 rabbits). The size of cages was: length=750 mm, width=460 mm and height=330 mm with a density of 17.4 rabbits/m² for the cages with 6 rabbits and 11.6 rabbits/m² for the cage with 4 rabbits. The animals have been randomly selected, however, being healthy and having an equivalent average live weight.

At 42 days of age the rabbits were batched into 6 groups of 6 rabbits (T₁, T₂, LPS100, LPS50, LPS75 and LPS150) with similar average live weight, and the experiment was conducted in two days (42 and 43 days of age) and then the rabbits were slaughtered at 44-45 days of age.

4. Main experiment

For the big part, the batching took into account the weight at weaning and the litter of origin of the kits. The mean weight and standard deviation was equivalent among the groups and the cages in order to avoid cages of “heavy animals” and cages of “light animals”. The litters were also spread to the different groups and cages in order to avoid an effect of litter of origin. Three hundred and twenty rabbits were divided into the four experimental groups DF100, ST100 DF75, ST75 of 80 rabbits each. Animals were housed in collective cages of 5 rabbits/cage and then 64 cages were used (16 cages/group). The size of cages was: length=750 mm, width=460 mm and height=330 mm, with a density of 14.5 rabbits/m².

For the small part, the batching was made in a similar way as the big part. To reduce the litter effect, one full litter/group was selected, with average weight and standard deviation similar to that of the selected population. A particular effort was made to reduce the weight variability in order to have comparable animals. Animals (48 rabbits divided into 12 rabbits/experimental group) were housed in individual digestibility cages. The size of cages was: length=500 mm, width=400 mm and height=310 mm, with a density of 5 rabbits/m². In this part of experiment we collected the faeces from 42 to 46 days of age (after one week of restricted feeding adaption).

5. Diets and feeding mode

The animals in the pre-experiment were fed a conventional fattening diet without antibiotics, containing 16.5 % crude protein, 11.55 % starch, 19.22 % cellulose and 2.5 % crude fat, during the entire experimental period (35 to 44/45 days of age). The animals were fed *ad libitum*.

For the main experiment, from weaning (35 days of age) to the end of the experiment (72 day of age for the big part) two diets without addition of drugs were used (coccidiostats or antibiotics). Digestible energy, crude fat, cellulose and crude protein levels were equivalent between diets ST and DF. The feed compositions differed in starch (16 % in ST vs. 11.86% in DF), in digestible fiber (17% in ST vs. 22% in DF), and hemicelluloses (10.82% in ST vs. 12.89% in DF). The two diets had a high level of fiber making them “secure” on a sanitary risk basis. Access to water was free. For the 2 restricted groups (ST75 and DF75) the animals were fed in one distribution in the morning between 7:30 and 8:30 hour.

5.1. Restricted feeding plan

To achieve the objective of a 75% quantitative restriction, we had a theoretical schedule according to rabbits age and feed intake. The feed intake of the 2 *ad libitum* groups was controlled for periods of 3 to 4 days in order to readjust the amount of feed to be distributed to the 2 restricted groups. This adjustment was realized independently in the both parts of main experiment. Thus, the feed intake levels of ST75 and DF75 were fixed to the 2 groups ST100 and DF100, respectively. In the big part, the mortality rate was considered to recalculate the total amount to be distributed in each cage.

6. Live performances measurements

6.1. Growth

The animals of the pre-experiment were weighed at 35 (weaning), 42 and 43 days of age to perform batching and set the amount of LPS administered to each animal.

In the main experiment the individual rabbit's weight was controlled at 35 (weaning), 42, 49, 63, 64 (mid and end of the period of restricted feeding), and at 71 days of age (end of the experiment) for the big part, at 35 (weaning), 42 and 46 days of age (end of the experiment) for the small part. The animals were weighed before the food distributions.

6.2. Feed intake

In the pre-experiment the feed intake was measured at weaning (35 days), 42 and 43 days of age.

In the main experiment the feed intake was measured by periods of 3 to 4 days during the restricted feeding period (35 to 64 days of age). After the restricted feeding period, consumption was monitored (refusal) only at 63 days of age and at 71 days of age.

7. Monitoring of health status

7.1. Mortality

During the main experiment, mortality was monitored daily (weekends included). The day and cause of the death was marked, as well as the weight of the rabbit. The causes of death were indicated: diarrhea, total paralysis, respiratory problems and others (f.e. broken leg).

7.2. Morbidity

During the pre-experiment the health status of animals was controlled at the weaning (35 days of age), at 42 and 43 days of age.

During the main experiment, , the control of the health status was performed at weaning (35 days of age) and at each weighing fixed at 35, 42, 49, 63, 64 and 71 days of age for the big part and at: 35, 42 and 46 days of age for the small part. Morbidity control involved the following aspects; general condition (posture. etc...), head (eyes, respiratory system: nasal discharge/nose blowing...) inner face of the front legs ("signs" of rhinitis), abdomen (palpation for detecting paralysis) and rear part of the body (diarrhea, mucus ...). Animals were then categorized: healthy, diarrhea, strong diarrhe, presence of mucus, caecal paresis, respiratory problems and other.

8. Slaughtering and samplings

8.1. Injections

8.1.1. LPS injections in pre-experiment and main experiment (just in the big group)

To induce a relatively acute inflammation, we chose to use an agent commonly used for this purpose, LPS (derived from *E. coli*, serotype O26: B6) solutions were prepared from lyophilized LPS (Sigma-Aldrich) dissolved and diluted to different concentrations in saline sodium chloride 0.9g per 100ml of solution and injected intraperitoneal. The injections were made with 10 ml syringes (accuracy of the injected dose of 5%) and needles of 0.6 mm diameter and 25 mm long.

The rectal temperature of the animals was recorded every 30 min from 1 hour before the injection to 6 hour after the injection. The rectal temperatures after 24 and 48 hours was also measured.

8.1.2. Pre-experiment

This experiment was aimed to determine which dose of LPS (50-200 µg/kg of BW) could cause a more uniform and measurable inflammatory response without having a lethal effect to be use on main experiment, and if these doses induce inflammation by a quantifiable increase in rectal temperature (fever). It was performed in 2 days:

Day 1: Evaluation of the impact of dose of 100µg LPS/kg of BW on the evolution of the rectal temperature of 6 rabbits of 42 days of age. A control group (T₁) of 6 rabbits was injected with saline at the same time.

Day 2: Depending on the effect of dose of 100µg LPS/kg of BW and rectal temperature, 3 doses of LPS (50, 75, and 150 µg/kg of BW) were selected to be tested on 6 rabbits 43 days of age per group (18 rabbits in total) and a control group (T₂) injected with saline at the same time.

8.1.3. Main experiment

Eight rabbits per group (32 rabbits in total) at 43 days of age were selected in the average weight intra-group. They received an intraperitoneal injection of 100µg LPS/kg of BW. This part of experiment aimed to determine the effect of the main dietary energy source and restricted feeding on inflammatory response of rabbits.

8.2. Euthanasia

Animals injected with LPS in pre-experiment and main experiment were euthanized 48h post-injection by electrical stunning followed by bleeding.

8.3. Sacrifices for main experiment (just for the big part)

The animals were sacrificed at 4 different ages 42, 49, 63 and 71 days (see experimental design schedule) at 2 p.m. To have the same mean and standard deviation in sacrifices and remaining animals in each group, one healthy animal/cage was chosen on the range of weight of the groups. We used the same method adopted to select the animals for the LPS injections. The animals were sacrificed using electrical stunning followed by bleeding.

8.4. Samplings in the main experiment

8.4.1. Diets and faeces (just in the small part)

The 2 diets were collected in separate plastic bag and sealed under *vacuum* for long-term preservation to be analyzed in laboratory. Collection of faeces was conducted on weekdays between 42 and 46 days of age (one week after the start of restricted feeding) for analysis of digestibility.

8.4.2. Tissues (just for the big part)

After slaughter the spleens and the caecal appendixes of the rabbits were removed and weighed. Caecal appendixes were emptied and washed with physiological water (0.9% NaCl) before weighing.

9. Chemical analyzes

9.1. Diets and faeces Analyses

The chemical analyses of the diets and faeces were carried out as follow: dry matter and organic matter.

We used a conventional method for analyzes of dry matter and organic amtter (European Group on Rabbit Nutrition Workshop of the concerted actions "ERAFE"). Dry matter was determined by heating for 24 hours at 103 °C. Weighing after 24 hours of drying (dry weight), and calculation of the difference between the dry weight and the fresh weight, expressed in percentage of dry matter.

Following the assay lab dry matter, the test samples were placed in the crucibles. A first temperature to 250 °C was carried out for 1 hour, then a second stage at 550 °C for 5 hours. We leave the crucibles from the oven and allowed to cool in a desiccator (20 min). Weighing crucibles and registered the calcined weight, to determine the organic matter.

10. Statistical analysis

The results of main experiment were analyses using the MIXED linear model SAS 9.2 (2008). Variance analysis was carried out to evaluate the effect of feed intake level (*ad libitum* vs. 75% of *ad libitum*), the energy source (starch vs. fibre) and the feed intake level x energy source interaction. The effect of the cage for the growth measurements is considered random. Concerning the sanitary status measurements, a categorical analysis using the CATMOD procedure was used. Differences between groups with $P < 0.05$ were considered statistically significant whereas those with $0.05 < P < 0.1$ were considered as tendencies toward differences.

The results of the LPS injection in pre-experiment and main experiment were performed with R statistical programme version 2.14.2 using “nlm”, “car” and “agricole” packages. Two way analysis of variance (ANOVA) for repeated measurments (in the same animals) was used. Differences between groups with $P < 0.05$ were considered statistically significant.

Chapter 3

Results

1. The results of main experiment

1.1. Live weight

The live weight at 35 days of age was not significantly different between groups. There was no effect of the energy source (ES) or the interaction between (ES) and intake level (IL) on the live weights of the animals all along the experiment (Table 1.1). Two week after the restriction strategy started (49 days of age) the weight was 13% lower in the restricted groups ($P<0.001$) compared to rabbits fed *ad libitum* (AL). At the end of restriction period (64 days of age) the live weight was 9% lower in the restricted groups ($P<0.001$) compared to AL rabbits, in spite of 25% less feed intake level. One week after re-feeding (71 days of age) the live weight was 5% lower in the restricted groups ($P<0.001$) compared to AL rabbits (Table 1.1).

Table 1.1: Live weight (g) of rabbits according to the dietary energy source and the intake level

Live weight at day	Groups ¹				RMSE	P-values		
	ST100	ST75	DF100	DF75		Energy Source(ES)	Intake Level(IL)	Interaction ES x IL
35 days	1038	1038	1038	1038	115	0.99	0.99	0.98
42 days	1416	1265	1412	1247	131	0.45	0.001	0.65
49 days	1779	1563	1775	1557	143	0.77	0.001	0.96
64 days	2497	2288	2486	2255	176	0.43	0.001	0.68
71 days	2805	2680	2804	2654	204	0.67	0.001	0.68

1: ST100=Starch *ad libitum*, ST75=Starch restricted at 75% of *ad libitum* intake, DF100=Digestible fibre *ad libitum*, DF75=digestible fibre restricted at 75% of *ad libitum* intake

RMSE: Root Mean Square Error

1.2. Daily weight gain

There was no effect of the energy source (ES) or the interaction ES x IL on the growth of the animals all along the experiment (table 1.2). At the end of restriction period (35-64 days) daily weight gain was 15% lower in the restricted groups ($P<0.001$), after one week of re-

feeding (64-71 days) it was 28% higher in the restricted groups ($P<0.001$) and during the whole experiment (35-71 days) was 8% lower in the restricted groups ($P<0.001$) compared to AL rabbits (table 1.2).

Table 1.2: Daily weight gain (g/day) of the rabbits according to the dietary energy source and the intake level

Periods	Groups ¹				RMSE	P-values		
	ST100	ST75	DF100	DF75		Energy Source (ES)	Intake Level (IL)	Interaction ES x IL
1 st restriction period (35-49)	53.3	37.7	52.7	36.6	4.6	0.16	0.001	0.71
2 nd restriction period (49-64)	47.1	48.3	46.5	47.3	5.2	0.34	0.26	0.84
Total restriction period (35-64)	50.3	43.0	49.6	42.0	3.9	0.22	0.001	0.80
Re-feeding after restriction (64-71)	43.9	59.6	45.9	56.0	7.3	0.55	0.001	0.09
Total period of experiment (35-71)	49.2	46.0	48.7	44.7	3.7	0.14	0.001	0.47

1: ST100=Starch ad libitum, ST75=Starch restricted at 75% of ad libitum intake, DF100=Digestible fibre ad libitum, DF75=digestible fibre restricted at 75% of ad libitum intake

RMSE: Root Mean Square Error

1.3. Daily feed intake

The intake level for ST restricted group was 78% of AL intake and for DF restricted group was 77% of AL intake; we did not obtain 75% of AL intake but the two restricted groups were sufficiently restricted and also in the same way (table 1.3).

The effect of energy source on the feed intake was calculated only for AL groups and not for restricted groups during the restricted periods (35-49, 49-64 and 35-64) as the restricted animals received the same amount of food. The effect of the energy source was not significant during the experiment. No effect of the interaction between energy source and intake level was observed either. Daily feed intake after one week of re-feeding (64-71 days) was 16% higher in the restricted groups ($P<0.001$) and 14% lower during the whole experimental period (35-71 days) in the restricted groups ($P<0.001$) compared to AL rabbits.

Table 1.3: Daily feed intake (g/day) of the rabbits according to the intake level and the dietary energy source

Periods	Groups ¹				RMSE	P-values		
	ST100	ST75	DF100	DF75		Energy Source (SE)	Intake Level (IL)	Interaction ES x IL
First restriction period (35-49) [§]	118.4	91.0	117.3	89.6	5.43	0.55		
Second restriction period (49-64) [§]	150.6	119.6	153.1	119.7	13.76	0.60		
Total restriction period (35-64) [§]	135.1	105.8	135.8	105.2	8.35	0.79		
Free feeding after restriction (64-71)	174.6	202.2	172.1	200.4	14.31	0.54	0.001	0.92
Total period of experiment (35-71)	142.7	124.6	142.9	123.7	5.25	0.79	0.001	0.73

§: The effect of diet (main dietary source of energy) is calculated only for AL groups during restriction period, as the restricted animals all receive the same amount of food, the intake level and interaction is not calculable.

1: ST100=Starch ad libitum, ST75=Starch restricted at 75% of ad libitum intake, DF100=Digestible fibre ad libitum, DF75=digestible fibre restricted at 75% of ad libitum intake

RMSE: Root Mean Square Error

1.4. Feed conversion

There was no effect of the energy source (ES) or of the interaction ES x IL on the feed conversion of the animals all along the experiment. Feed conversion after restricted period (35-64 days) and after free feeding (64-71 days) was 10% lower in the restricted groups ($P < 0.001$); during whole experimental period it was 7% lower in the restricted groups ($P < 0.001$) compared to AL rabbits.

Table 1.4: Feed conversion ratio of the rabbits according to the dietary energy source and the intake level

Period	Groups ¹				RMSE	P-value		
	ST100	ST75	DF100	DF75		Energy Source (ES)	Intake Level (IL)	Interaction ES x IL
First restriction period (35-49)	2.25	2.44	2.28	2.48	0.101	0.47	0.001	0.38
Second restriction period (49-64)	3.50	2.59	3.35	2.51	0.248	0.79	0.001	0.37
Total restriction period (35-64)	2.80	2.52	2.76	2.50	0.159	0.50	0.001	0.76
Free feeding period (64-71)	3.94	3.47	3.92	3.63	0.414	0.48	0.001	0.40
Total period of experiment (35-71)	2.95	2.74	2.95	2.77	0.114	0.70	0.001	0.66

1: ST100=Starch *ad libitum*, ST75=Starch restricted at 75% of *ad libitum* intake, DF100=Digestible fibre *ad libitum*, DF75=digestible fibre restricted at 75% of *ad libitum* intake

RMSE: Root Mean Square Error

1.5. Spleen and appendixes

There was no effect of the energy source (ES) or the interaction ES x IL on the weight of spleen and appendixes of the animals all along the experiment; nor for the intake level effect on spleen weight, but there was a tendency after one week re-fed ($P=0.051$) in the restricted groups and the spleen weight of the animals was 6% higher in these groups compare to AL rabbits. The weight of appendixes after restricted period (at 63 days) was 7% lower in the restricted period ($P<0.05$) compared to AL rabbits.

Table 1.5: Spleen and appendix weight[§] of the rabbits according to the dietary energy source and the intake level

Age (days)	Organs (g)	Groups ¹				RMSE	P-values		
		ST100	ST75	DF100	DF75		Energy Source (ES)	Intake Level (IL)	Interaction ES x IL
42	Spleen	0.89	0.92	0.99	0.87	0.15	0.70	0.37	0.13
	appendixes	3.53	3.86	3.96	3.86	0.36	0.074	0.32	0.075
49	Spleen	0.78	0.89	0.83	0.85	0.13	0.88	0.11	0.31
	appendixes	3.91	4.04	3.74	3.72	0.45	0.096	0.70	0.60
63	Spleen	1.75	1.79	1.73	1.81	0.18	0.94	0.32	0.74
	appendixes	4.01	3.76	4.03	3.69	0.44	0.84	0.042	0.74
71	Spleen	1.48	1.60	1.50	1.57	0.14	0.98	0.051 [#]	0.64
	appendixes	4.87	4.77	4.36	4.92	0.90	0.52	0.42	0.25

§: The weights of the organs are related to the body weight to cancel the effect of live weight on organ's weight.

1: ST100=Starch *ad libitum*, ST75=Starch restricted at 75% of *ad libitum* intake, DF100=Digestible fibre *ad libitum*, DF75=digestible fibre restricted at 75% of *ad libitum* intake. The values are means of 10 rabbits per group.

RMSE: Root Mean Square Error

1.6. Mortality, morbidity and health risk index

Mortality (1.6%) and morbidity (6.7%) rate were very low during the experiment. The energy source and intake level had no significant effect on mortality (0% for the total period in restricted groups and 3.2% for the total period in AL groups) nor morbidity (8.2% for the total period in restricted groups and 5.2% for the total period in AL groups) nor sanitary risk index (the sanitary risk for the total period in restricted groups was of 8.2% and 8.4% in AL groups). The 3 cases of mortality observed were caused by digestive disorders (diarrhea). q

Table 1.5: Mortality, morbidity and Health risk index (mortality + morbidity)

Periods (days)	Groups ¹				
	ST100	ST75	DF100	DF75	Total
1st restriction period (35-49) (%)	0%	0%	0%	0%	0%
n. of dead animals/total	0/80	0/80	0/80	0/80	0/320
2nd restriction period (49-64) (%)	0%	0%	4%	0%	1%
Mortality n. of dead animals/total	0/50	0/50	2/50	0/50	2/200
Re-feeding period (64-71) (%)	2.5%	0%	0%	0%	0.6%
n. of dead animals/total	1/40	0/40	0/38	0/40	1/158
Total period (35-71) (%)	2.5%	0%	4%	0%	1.6%
n. of dead animals/total	2/80	0/80	3.2/80	0/80	5.2/320
1st restriction period (35-49) (%)	1.2%	2.5%	1.2%	2.5%	1.8%
n. of morbid animals/total	1/80	2/80	1/80	2/80	6/320
2nd restriction period (49-64) (%)	4%	4%	2%	0%	2.4%
n. of morbid animals/total	2/50	2/50	1/50	0/50	5/208
Morbidity Re-feeding period (64-71) (%)	0%	5%	5.2%	2.5%	4.8%
n. of morbid animals/total	0/40	2/40	2/38	1/40	5/104
Total period (35-71) (%)	3.2%	11.5%	7.2%	5%	6.7%
n. of morbid animals/total	2.6/80	9.2/80	5.8/80	4/80	21.6/320
1st restriction period (35-49) (%)	1.2%	2.5%	1.2%	2.5%	1.8%
n. of dead and morbid animals/total	1/80	2/80	1/80	2/80	6/320
2nd restriction period (49-64) (%)	4%	4%	6%	0%	3.3%
Health risk index n. of dead and morbid animals/total	2/50	2/50	3/50	0/50	7/208
Re-feeding period (64-71) (%)	2.5%	5%	5.2%	2.5%	5.7%
n. of dead and morbid animals/total	1/40	2/40	2/38	1/40	6/104
Total period (35-71) (%)	5.7%	11.5%	11.2%	5%	8.3%
n. of dead and morbid animals/total	4.6/80	9.2/80	9/80	4/80	26.8/320

1: ST100=Starch *ad libitum*, ST75=Starch restricted at 75% of *ad libitum* intake, DF100=Digestible fibre *ad libitum*, DF75=digestible fibre restricted at 75% of *ad libitum* intake

1.7. Digestive efficiency (the small part of main experiment)

There was no effect of the energy source or the interaction ES x IL on the mean live weight of animals, dry matter and organic matter apparent digestibility during the restricted period in the digestive experiment (Table 1.7). There was no significant effect of the energy source on the daily feed intake of AL fed animals. The live weight was 12% lower in the restricted groups ($P < 0.001$) compared to rabbits fed AL. The dry matter apparent digestibility was 2% higher in restricted groups ($P < 0.05$) compared to rabbits fed AL. There was no significant difference in organic matter apparent digestibility between restricted and AL fed groups.

Table 4.1: Whole tract digestive efficiency according to the main dietary source of energy and the intake level during the feed restriction (from 42 to 46 days of age)

	Groups ¹				RMSE	P-values		
	ST100	ST75	DF100	DF75		Energy Source (ES)	Intake Level (IL)	Interaction ES x IL
Live weight (g)	1541	1358	1524	1365	55	0.79	0.001	0.49
Feed intake (g/day) ^f	143.0	108.0	140.0	109.0	10.4	0.51		
Dry matter apparent digestibility (%)	63.94	65.65	63.62	64.89	2.1	0.41	0.05	0.74
Organic matter apparent digestibility (%)	64.20	65.28	64.22	64.69	2.4	0.69	0.30	0.68

^f: The effect of feed (source of energy) is calculated only for AL groups and not for restricted groups, as the restricted animals all receive the same amount of food, the intake level and interaction is not calculable.

2. The results of LPS injection in pre-experiment

The animals showed a rise in rectal temperature over time, which means that the animals expressed fever in all groups. All doses led to a rise in rectal temperature (a minimum of 0.5 °C). The group with 100 µg/kg of BW of LPS had a higher response (1.1 °C) in terms of temperature and it was different from all other groups. Therefore, we chose this dose of LPS to use in the main experiment (big part).

Table 2.1: Rectal temperature for determining the dose of LPS to use in the pre-experiment

	LPS ($\mu\text{g}/\text{kg}$ of BW) [§]						RMSE	P-values			
	150	100	75	50	0 (T ₁) [#]	0 (T ₂) [#]		Time	Time ²	Dose	Time:dose
Mean of rectal T°C	39.8 ^{b,d}	40.1 ^b	39.3 ^{a,d}	39.5 ^{a,d}	38.9 ^c	39.0 ^{a,c}	0.218	***	***	***	*

#: T₁ and T₂ are the control groups with 0 $\mu\text{g}/\text{kg}$ of BW of LPS injected

§: the values are means of 6 rabbits per group

3. The results of LPS injection in main experiment

The LPS injection led to a rise on body temperature in all groups but the groups did not have a significant effect on the response of the animals in terms of fever (Table 1.3).

Table 3.1: Rectal temperature over time for rabbits subject to a LPS injection, and according to the dietary energy source and the intake level

	Groups [‡]				RMSE	P-values		
	ST100	ST75	DF100	DF75		Time	Time ²	Groups
Mean of rectal T°C	39.8	39.8	39.7	40.2	0.232	ns	***	ns

‡: 100 $\mu\text{g}/\text{kg}$ of BW of LPS injected for all groups and the values are means of 8 rabbits per group

RMSE: Root Mean Square Error

Chapter 4

Discussion

1. The effect of feed restriction on growth, feed intake and digestive efficiency

The growth was reduced during feed restriction as expected (Boisot *et al.*, 2003; Bergaoui *et al.*, 2008; Gidenne *et al.*, 2009a, b) and a compensatory growth was observed when the previously restricted rabbits were re-fed *ad libitum* (Lebas and Laplace, 1982; Ledin, 1984a, b; Tumava *et al.*, 2002; Dalle Zotte *et al.*, 2005; Matics *et al.*, 2008; Gidenne *et al.*, 2009c). The impact of an intake limitation on weight gain was more severe at the first week of restriction period (just after weaning) than after, as previous observed (Martignon *et al.*, 2010a; Gidenne *et al.*, 2009c). The feed intake level was increased moderately in the restricted groups during the re-feeding period but we did not observe any overeating in restricted groups, as previous studies (Gidenne and Feugier, 2009; Taranto *et al.*, 2003). The moderate increase of the feed intake level of the restricted animals during the re-feeding period could be explained by the digestive physiology and feeding behavior of the rabbit which has a small stomach (30% of the whole digestive tract) and adapted to numerous meals (Gidenne and Lebas, 2006). We can associate these effects of feed restriction on growth and feed consumption to the improvement of the feed conversion ratio during the re-feeding period.

Gidenne *et al.* (2009), in a big experimental design (496 rabbits/treatment), found a compensatory growth during the re-feeding period (after 54 days of age) and an improvement in feed conversion in the restricted groups (with 80%, 70% and 60% of *ad libitum* (AL) intake). Dalle Zotte *et al.* (2005), observed a compensatory growth in the rabbits restricted by 70% and 90% of the AL intake from 5 to 8 weeks of age and then rationing from 8 to 11 weeks of age, and the compensatory growth was correlated with a better feed conversion. These results were in agreement with our study.

During feed restriction the dry matter (DM) digestibility was increased by 2% and we expected to find a better organic matter (OM) digestibility too. But few studies deal with an increase in OM digestibility. Gidenne and Feugier (2009) showed that the OM digestibility was not significantly affected by restricted feeding (80, 70 and 60% of AL) after 7 days of application of a restriction strategy. Tumova *et al.* (2007) had the same results in restricted fed rabbits (60% of AL) between 42 to 49 days of age. Diaz Arca *et al.* (1999), did not find a significant effect of feed restriction on OM digestibility (digestibility was calculated from 45 to 60 days of age) without a delay of adaption in restricted animals even with a reduction of 10% of intake level.

According to these studies, to observe an improvement in OM digestibility, more than one week adaption period to the feed restriction strategy is needed.

2. The effect of restricted feeding on health and immunity

The primary goal of our experiment was to obtain original data on digestion and immunity. However, animals exhibited a very low mortality level and any significant effect of the experimental treatments on rabbit health status was observed. Gidenne *et al.* (2009a), showed a reduction by a half of mortality and morbidity in restricted animals (60, 70 and 80% of AL) during the restriction period between 35 to 54 days of age with 496 rabbits/group; they obtained the same results from 63 to 71 days of age (re-feeding period) in the restricted groups (75% of AL) with 503 rabbits/group (Gidenne *et al.* 2009b). Szendrő *et al.* (2008), observed less mortality in restricted rabbits (80% of AL) from 35 to 84 days of age (40 rabbits/group). These studies are not in agreement with our results. Probably the limited number of animals we used, the very good environmental condition of the experimental rabbitry, and the very “safe” feed (both rich in fibre) distributed in ST and DF groups led to a very low mortality and morbidity in all the groups.

Restricted feeding led to a lower weight (-7%) ($P < 0.05$) of the empty appendix in rabbits at the end of restricted period (64 days of age) and showed a tendency (+6%) ($P = 0.051$) on the weight of the spleen after one week re-feeding (71 days of age) in restricted groups compare to AL ones. In a previous study at INRA on rabbits, Martin *et al.* (2012, Master Thesis), also found a lower weight of the appendix in the restricted groups (70% of AL) and no effect of restricted feeding on the weight of the spleen at 64 days of age. In another study conducted at INRA (personal communication), with restriction level at 75% of AL with 40 animals per group (at 42 d, 49 d, 63 d and 71 days of age) was observed any effect of restricted feeding on appendix and spleen weights. Oliveira *et al.* (2013), did not observed any effect of restricted feeding on the weight of internal organs in rabbits restricted from 33 to 40 days of age and from 54 to 61 days of age. These studies are part in agreement with our study.

Abe *et al.* (2001), in a study on mice with dietary restriction at 50% of the AL for 6 months found that the entire number of T-lymphocytes was not affected by restricted feeding. This result could explain the absence of a significant effect of restricted feeding on the weight of the spleen in our study. The size of empty appendix is probably affected by feed intake level, but the contrasting results suggest to investigate further in this topic.

Injection of LPS (50, 75, 100 and 150 $\mu\text{g}/\text{kg}$ BW) led to a rise in body temperature (0.5-1.1 $^{\circ}\text{C}$) in rabbits in the pre-experiment. MacDonald *et al.* (2011), with calorie restricted mice (75 and 50% of AL for 28 days) and injected with 50 $\mu\text{g}/\text{kg}$ BW of LPS on day 29, observed a shorter fever in duration in 75% of AL restricted mice respect of the control group. Brito *et al.* (1995), with an intravenously injection of LPS (50 and 100 $\mu\text{g}/\text{kg}$ BW) in rabbits fed with three different diets for 8 weeks (based on the effect of cholesterol) had fever (39 ± 1.3 $^{\circ}\text{C}$) after the LPS injection. These results showed the effect of LPS injection on body temperature rise and they are in agreement with our study.

Diet and intake level had no effect on the response to an LPS injection. We chose 43 days of age (one week after restricted feeding started) for injection of LPS, as the effect of feed restriction on health (mortality and morbidity) is visible at the beginning of the feed restriction; however we did not see any effect on our measurement of the inflammatory response in the rabbits. In mice the effect of 3-4 months feed restriction on the inflammatory response and the T-lymphocytes function was studied, but any effect was observed (Jolly *et al.*, 1999; Jolly *et al.*, 2001). Thus, in order to observe a significant effect of restricted feeding on the inflammatory response, it's suggested to consider a longer restriction period.

3. The effect of dietary energy source

The two diets were formulated to reach a similar level of digestible energy (DE) (2415 kcal DE/kg) and a high level of ADF (18%). DE was adjusted between the two diets with the level of digestible fibre (pectin and hemicelluloses): 22% in DF diet respect to 17% in ST diet, and reversely for starch. The energy source (ST or DF) had no significant effect on growth, feed intake, health or digestibility.

Gidenne and Jehl (1996), with two different diets (digestible fibre/starch ratio from 0.65 to 1.99) fed 28 – 72 days old rabbits, showed the feed intake, weight gain and OM digestibility were not significantly affected by the diets. Gidenne and Perez (2000), where showed the effect of digestible fibre replacement (25 to 15%) by starch (12 to 23%) digestible in four different diets (ADF = 18%) with decreasing digestible fibre/starch ratio (2.08, 1.37, 0.95, and 0.62) didn't observe any significant difference on feed intake, weight gain and feed conversion ratio between different groups and OM digestibility was similar between two extreme diets; the digestible fibre replacement by starch did not change the digestive efficiency of the rabbit. Gidenne and Bellier (2000), in growing rabbits with three experimental diets to obtain a variation in the level of "digestible" fibre: a control, and two diets where a portion of starch was substituted either by hemicellulose (HC) or by hemicellulose + pectins (HC + P), all three diets had a similar low-digestible fibre (lignocellulose, ADF); showed the digestibility was similar between groups.

The lack of effect of energy source on growth and digestive efficiency in our study are in agreement with these previous literatures.

In the second part of study of Gidenne and Perez (2000), with a total of 2328 rabbits fed from post weaning to slaughter, the mortality was increased (+5.5%) by decreasing digestible fibre/starch ratio in two extreme diets for the whole period (Perez *et al.*, 2000). Soler *et al.* (2004), with 5 different diets in digestible fibre/starch ratio (from 0.77 to 3.53) distributed in a total of 4,000 rabbits fed from 17 to 43 days of age and then switched to a

commercial feed to 63 days of age, showed the mortality rate was significantly reduced increasing digestible fibre/starch ratio. Tazzoli *et al.* (2009), fed five different diets with increased digestible fibre to starch ratio (from 1.0 to 1.9) to 250 rabbits from 27 to 76 days of age and they did not observe any significant effect of DF on health status (mortality and morbidity), replacing starch with DF improved feed efficiency without changing caecal fermentation activity.

The absence of statistically significant differences of sanitary index in our study are probably attributable to the “safer” range in the digestible fibre/starch ratio (1.06 to 1.86, in ST and DF diets, respectively), confirming what observed by Tazzoli *et al.* (2009).

Conclusions

The current problems in rabbit breeding are high morbidity and mortality rate because of digestive disorders after weaning. The use of antibiotics (given under veterinarian prescription) helps to control these disorders. But this practice is questioned by consumers and society. Thus, rabbit industry decided to reduce the use of antibiotics. Several factors can have an effect on the health status (hygiene, breeding in batch, nutrition and feed strategy). In our study as we had just 3 died rabbits we are not able to suggest the benefits of feed restriction or high digestible fibre diet to improve the health status of young rabbits. But the control of feed intake level and its relationship with digestive disorders was showed in other studies and could be a solution to reduce the morbidity and mortality rate in rabbit production.

However the restriction strategies should be adapted according to the objectives of farmers: health status improvement and/or reduction of feed costs. So feed restriction strategy could lead to a reduction of feed consumption and an improvement of health status of rabbits. Moreover, the cost of feed is very variable and regularly increases. Thus improving the feed conversion through feed restriction strategies would therefore be a favorable way to reduce the feed cost. In our study the feed conversion was improved in restricted groups to AL ones during the re-feeding period. Thus a reduce of feed consumption and an improvement of feed conversion could lead to a minor feed cost for farmers.

Replacing a part of starch with digestible fibre (digestible fibre/starch ratio from 1.06 to 1.86) had no effect on different functions of rabbits. We cannot suggest the use of the higher digestible fibre diet.

The inflammatory response of rabbits to injection of 100 µgLPS/kg of BW (in terms of fever) was confirmed in our study. In order to see a significant effect of feed restriction probably a longer restricted period was needed. The effect of dietary energy source on rabbit's inflammatory response in terms of fever needs more studies.

The feed restriction led to a lower appendix weight and showed a tendency in a higher spleen weight in rabbits. The effect of feed restriction and dietary energy source on the weight of appendix and spleen need to be study more to confirm their beneficial effects on rabbit's digestive immune system.

Acknowledgements

I would like to thank Christelle Knudsen, for the total support and assistance she gave me during my work at INRA.

Thanks also to professor Antonella Dalle Zotte and Doc. Thierry Gidenne always present and available.

I want to thank my family: my mum, my dad and my valiant brother Danial, for their support and endless patience; without them my project would not have been possible. A special thanks also to Anita and my adorable Sofia.

I want to thank all people who helped me during my project at INRA: Doc. Laurance Fortun-Lamothe, Doc. Sylvie Combes, Doc. Laurent, Vincent, Viviane, Carole, Murielle, Beatrice, and the staff of INRA's rabbitary.

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