The role of dietary carbohydrates in digestive disorders of rabbits

Reponen, Rebecca Aurora

Fi

THE UNIVERSITY OF ZAGREB

FACULTY OF VETERINARY MEDICINE

INTEGRATED UNDER-GRADUATE AND GRADUATE UNIVERSITY STUDIES IN *VETERINARY MEDICINE*

DIPLOMA THESIS

Rebecca Reponen

The role of dietary carbohydrates in digestive disorders of rabbits

Zagreb, 2024

Rebecca Reponen

Department of Animal Nutrition and Dietetics
Head of department: Prof. Hrvoje Valpotić, PhD

Mentor name: Prof. Tomislav Mašek, PhD

Members of the Committee for Defense of the Master's Thesis

1. Prof. Hrvoje Valpotić, PhD, Department of Animal Nutrition and Dietetics
2. Assis. Prof. Diana Brozić, PhD, Department of Animal Nutrition and Dietetics
3. Prof. Tomislav Mašek, PhD, Department of Animal Nutrition and Dietetics
4. Assoc. Prof. Kristina Starčević (substitute), Department of Chemistry and Biochemistry

The paper contains 29 pages, 5 figures, 2 tables, 20 literature citations.

Acknowledgements

I want to thank my mentor, Professor Tomislav Mašek, for the opportunity to work with him on this thesis and for his guidance throughout the process.

I want to dedicate this paper to my mom, dad, and brother for being the most loving support system throughout my studies, and for always believing in me.

CONTENTS

1. INTRODUCTION

Plant carbohydrates can be classified according to the complexity of their structure. In animal nutrition, carbohydrates primarily serve as a source of energy for normal life functions. In rabbits, carbohydrates can be digested and absorbed from the stomach and small intestine or fermented by the microflora of the cecum. Simple monosaccharide sugars such as glucose, fructose and galactose are absorbed by the small intestine in a similar way to other animal species. Starches are polysaccharides that are abundant in plants and are broken down to simple sugars during digestion. The age of the rabbit, the amount of food and the type of starch affect digestion and absorption in the small intestine of the rabbit. Starch that is not digested and absorbed in the small intestine reaches the cecum as a substrate for bacterial fermentation and can cause digestive disorders. Dietary fiber is defined as the part of the ingested feed, mainly the plant cell wall, that cannot be broken down by the intestinal enzymes of monogastric animals. Plant cell walls consist of complex carbohydrates such as cellulose, hemicellulose and pectins embedded in a lignin matrix. Nevertheless, most of these molecules can be broken down by bacterial fermentation in the cecum. The rabbit, as a strict herbivorous animal, is usually fed with diets containing at least 40% to 50% fiber. The importance of fiber in the diet is due to its effects on intake, rate of passage and role as substrate for cecal microflora. However, for the growing rabbit, one of the main challenges is to meet the fiber recommendations to prevent digestive troubles and impairment of performance. Digestive pathology of the growing rabbit is a major cause of losses after weaning. In commercial rabbit farms, the mortality rate from digestive disorders such as colibacillosis and rabbit enteropathy has ranged from 7% to 8% for the past 5 years. Moreover, digestive disorders are responsible for important morbidity characterized by growth depression and poor feed efficiency.

2. REVIEW OF THE RESULTS OF PREVIOUS RESEARCH

2.1. Rabbit digestive tract

The digestive tract of the rabbit consists of the stomach, small intestine, cecum and colon. The stomach of the rabbit comprises about 15% of the total volume of the digestive tract and is always partially filled. Though the pH may vary depending on the region of the stomach, it is usually very acidic in adult rabbits at a pH of 1-2. On the contrary, the pH of the suckling rabbits is around 5-6.5, to allow bacteria to pass through and form a healthy microbiota in the hindgut. The pH of the stomach drops quickly after weaning. The duodenum lies in such an angle to the stomach that compressive blockage of the pyloric valve may occur due to increased abdominal pressures such as gas, hepatomegaly or trichobezoars.

The small intestine follows the stomach and is on average 3 meters long with similar functions as in most other mammals, including secretion of bile, digestive enzymes and buffers. In contrast to other mammals, the small intestine is much shorter and makes up for only about 12% of the digestive tract.

Rabbits are hindgut fermenters, meaning that they possess a significant microbial flora in their cecum that performs fermentation to provide additional energy and nutrients. This microbial flora consists primarily of the genera *Bacteroides*, *Bifidobacterium, Clostridium*, *Streptococcus* and *Enterobacter*. The cecum has a weak muscular layer, and its contents are high in dry matter. The pH of the cecum is slightly acidic, at 5.4-6.8.

The colon of the rabbits is divided into proximal and distal, with the proximal colon consisting of three separate segments that differ from each other by the presence or absence of taenia and haustra. Gut-associated lymphoid tissue, as well as goblet and paneth cells, are also of particular importance in the colon, producing mucus and antimicrobial peptides. The colon contains an important “pacemaker” composed of ganglion aggregates that regulate peristalsis and excretion of hard and soft feces (SOHN and COUTO, 2012).

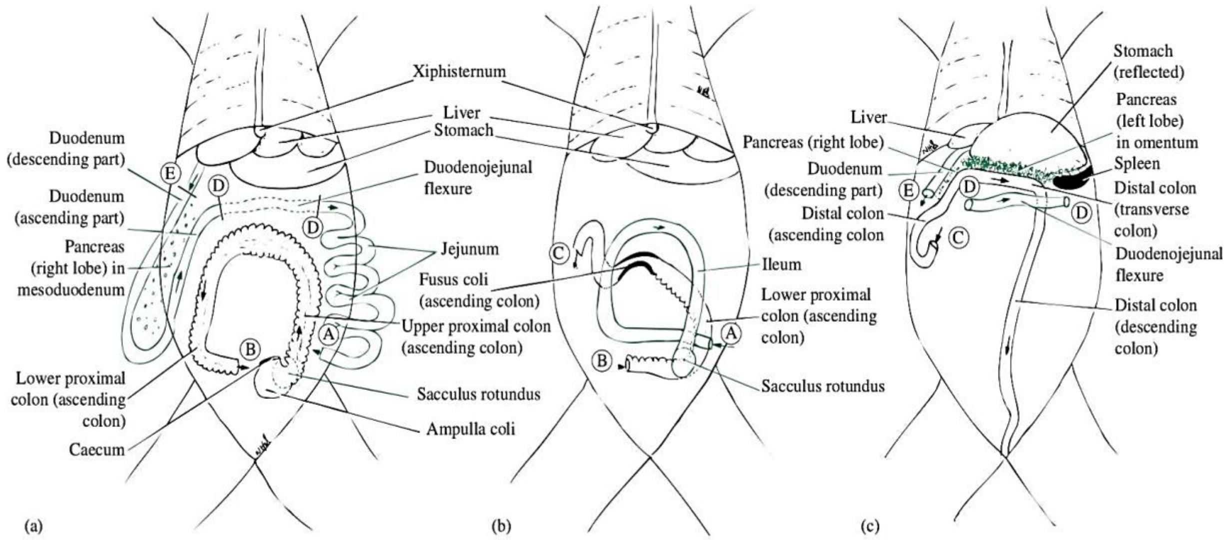

Figure 1. With permission from: SOHN and COUTO, 2012.

Rabbits engage in cecotrophy, which is the consumption of cecotropes that are produced by the process of hindgut fermentation. Cecotrophy takes place primarily during the day, while feeding and expulsion of hard feces occurs during the night. In the process of 'hindgut fermentation', food passes through the esophagus, stomach and small intestine, and finally into the colon. Nutrients are not effectively absorbed during this initial digestion process. Reverse peristalsis pushes food back into the cecum, and simple sugars are produced by bacterial fermentation.

Once the cecotrope is excreted, it will be re-ingested without chewing. The cecotrope is covered by a mucous layer that acts to protect it from stomach acids. It is important to establish that cecotrophy is not an attempt on behalf of the rabbit to correct a nutritional imbalance, but a defined digestive strategy to utilize complex carbohydrates. Cecotropes, in comparison to hard feces, have a higher protein, mineral and vitamin content, while hard feces are higher in fiber.

As rabbits are a prey species, a rapid feed transit time is important to keep their weight low for fast movement and escape from predators. Their transit time ranges between 3-9 hours, with 4-8 hours being the average. The transit occurs the fastest within the small intestine, at around 10-20 minutes. The cause of the wide variability in transit time could be effects of different markers used in the measuring of transit time, route of administration of the marker, or time of application of the marker. Mean retention time in rabbits ranges from 9-30 hours, but increasing dietary fiber stimulates cecocolic motility.

2.2. Diet composition

Sugars are distinguished as simple sugars and oligosaccharides, due to the differences in their digestion processes and their complexity. Glucose and fructose are the primary sugars found in rabbit feed, and exist either as their respective monosaccharides, or as the disaccharide sucrose. Glucose and fructose are effectively absorbed in the small intestine. Other disaccharides present in rabbit feed include maltose, consisting of two glucose molecules and derived from starch hydrolysis, and melibiose consisting of galactose and glucose, derived from root plants.

Oligosaccharides present in the rabbit feed include maltotriose, which consists of three glucose molecules and is derived from starch hydrolysis, and A-galactosides, which are a group of oligosaccharides that are not digestive by rabbit digestive enzymes and are thus effectively fermented by the microflora of the cecum. These A-galactosides are derived from pea and lupin seeds, or soybean meal.

Starch is a polysaccharide present in green plants and is considered the one of the most plentiful carbohydrates, second only to cellulose. Starch is a reserve polysaccharide made up of D-glucose units and exists in a granular form in nature. The shape and size of the granules may vary depending on its source, and the granule itself is made up of crystalline and amorphous components. Digestion of the starch granule occurs when enzymes are adsorbed onto the granule, and through erosion or penetration, hydrolysis occurs. Starch consists of chains of amylose and amylopectin, which consist of linear and branched glucose links, respectively. The proportion of amylose and amylopectin ranges depending on different plants, and this change in proportion has an impact on its digestibility. Rabbits excrete almost no starch in their feces, due to their ability to digest the polysaccharide nearly completely. The amount of starch in feces can vary slightly from 2% to 10% of intake, depending on the starch source and the age of the rabbit. The digestion of starch primarily occurs in the small intestine, though the stomach and large intestine contribute to its degradation in a smaller part as well. Starch digestion thus begins in the stomach, though specific and reliable measurements of the extent of gastric starch degradation have not been taken. It has been suggested that up to 30% of the starch ingested from the feed were degraded in the stomach, though 30% may be an overestimate. The digestion of starch occurs primarily in the small intestine under the action of pancreatic amylase. Other enzymes such as maltase and amyloglucosidase are also important. The activity of these digestive enzymes increases significantly during the weaning period. Starch that is not digested in the small intestine is transported to the cecum and undergoes fermentation by the microflora, producing volatile fatty acids and lactate. Rabbits are far more susceptible to digestive disorders during the weaning period, due to the copious changes occurring during this time. Previously it was believed that a high concentration of starches may increase the prevalence of digestive disorders during the weaning period, though more recent studies have revealed that starch intake has a relatively low effect on digestive disorders during this period. Therefore, the most significant dietary element in regard to digestive disorders during the weaning period is fiber intake. Due to the recent suggestion that fiber plays a far more significant role in the development of digestive disorders than starch, it is not the simple concentration of starch that should be considered critical, but rather the starch to fiber ratio, and the meeting of minimum fiber requirements. The intake of starch and fiber are linked in such a way that when starch content increases in the diet, fiber content tends to decrease. Thus, it is important to maintain the fiber above the requirements, at the expense of starch content (DE BLAS and WISEWAN, 2020).

As rabbits are monogastric hindgut fermenters and herbivores, a high-fiber diet is a requirement. Fiber should be the main component of the diet of the rabbit and can account for up to 50% of their diet. Fiber plays a critical role in rabbit digestive health, as it maintains a proper rate of passage of digesta through the digestive tract, aids mucosal function, and acts as a substrate for the gut microflora. ‘Dietary fiber’ is regarded as the parts of the feed that are not digested by the enzymes of the rabbit but can be fermented by the microflora. Thus, the term ‘dietary fiber’ may include not only plant cell walls, but also starches, oligosaccharides and even proteins associated with PCWs. The components of dietary fiber can be divided into two main groups based on where they are found, their chemistry, and properties: cell wall components, which are water-soluble non-starch polysaccharides and water-insoluble polymers, and cytoplasm components, which include oligosaccharides, fructans and starches. ‘Plant cell walls’ are polysaccharides associated with other molecules such as glycoproteins, phenolic compounds, acetic acid and lignin. Soluble fiber, or fermentable fiber, is the fraction of dietary fiber that is water-soluble. Representatives of this group include beta-glucans, oligosaccharides and resistant starches (KAY, 1982). In most mammals it is primarily fermented by the colonic microflora, which produces short-chain fatty acids such as acetate, propionate, and butyrate, as well as gas. These short-chain fatty acids provide energy after absorption, particularly for the epithelial cells that line the colon. However, in the rabbit cecal fermentation of fiber is particularly significant. Insoluble fiber is not water-soluble and cannot be broken down by the endogenous enzymes of the rabbit. Representatives include cellulose and lignin. Cellulose is the primary structural polysaccharide of plants and is only soluble in very acidic solutions. Lignin is a non-carbohydrate and adds the function of rigidity to the plant cell wall. Insoluble fiber increases mucus production in the large intestine, which promotes the movement of digesta. An increased crude fiber concentration aids in intestinal motility and reduces the overall nutritive value of the feed. To express fiber content in rabbit feed, we use the units neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude fiber (CF). NDF includes hemicelluloses, cellulose and lignins, while ADF includes cellulose and lignins and ADL includes lignings only. Crude fiber consists mainly of cellulose, with some lignins. Because of the complexity of dietary fiber, there is no method that can effectively quantify it, and can only be properly measured by the animal’s digestive process (DE BLAS and WISEWAN, 2020).

The digestive diseases and problems of rabbits are inherently linked to their diet. It is particularly important to understand the role of digestible and indigestible, or soluble and insoluble fiber. When we use the term ‘digestible’ in rabbits, we are not only referring to the digestion by the stomach and small intestine, but also degradation of fiber by the cecal microflora. The digestibility of fiber depends on its chemical structure and size. Larger, more indigestible fiber does not enter the cecum, and is instead excreted as hard feces. However, this indigestible fiber plays a critical role in stimulating intestinal motility. A diet lacking sufficient indigestible fiber results in reduced gut motility and reduced entry of digesta into the cecum. A low concentration of indigestible fiber also reduces appetite in rabbits. Lack of indigestible and digestible fiber may alter the proportions of volatile fatty acids produced, which in turn affects the cecal pH and gut motility.

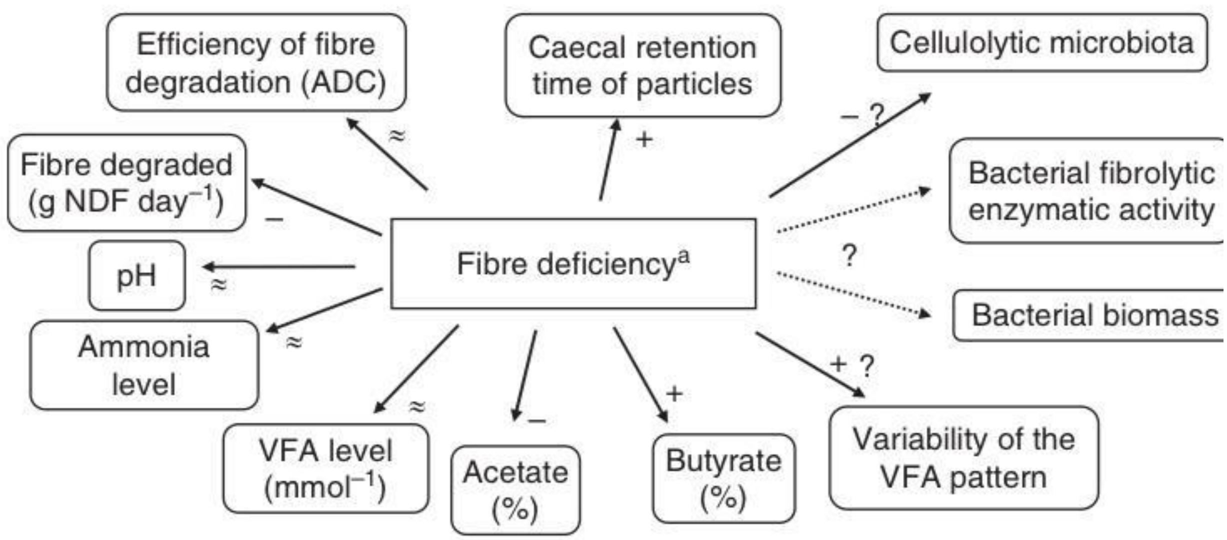

Figure 2. The possible effects of fiber deficiency in the rabbit diet.

2.3. Recommended diet

In terms of an ideal diet for commercial rabbit rearing, the most critical parameters are the minimum concentrations of fiber and the maximum concentrations of starch. Particularly levels of lignin should meet the minimum requirements along with long fiber.

Table 1. Selected recommended nutrient levels for intensively reared rabbits (g/kg food).
From: DE BLAS and WISEWAN, 2020.

Nutrient	Breeding does	Fattening rabbits	Mixed feed
Neutral detergent fiber	310-355	330-350	320-340
Acid detergent fiber	165-185	180-200	160-180
Crude fiber	140-150	150-160	145-155
Acid detergent lignin	55	50	55
Soluble neutral detergent fiber	Free	115	80
Starch	160-180	140-160	150-170

Similarly to commercially reared rabbits, the diet of pet rabbits needs to meet the minimum requirements for fiber to ensure proper gut motility and microfloral balance. Indigestible fibers like lignocellulose provide insoluble bulk that aids intestinal motility, and a diet too low in indigestible fibers can result in a variety of gastrointestinal disorders and predisposes the rabbit to proliferation of potential intestinal pathogens. An increased retention time occurs when crude fiber is below 120g/kg. The physical structure of the fibers in the diet is also important, as it has been demonstrated that finer ground diets with screen sizes of 1 increased intestinal retention time and cecal contents. With screen sizes of 2-7mm, digestive problems did not occur.

Starch levels above 150g/kg in the cecum have been shown to result in digestive upset in rabbits, particularly in younger rabbits that are more sensitive to starch overload (DE BLAS and WISEWAN, 2020).

Table 2. Selected recommended nutrient levels for pet rabbits (used with permission).
From: DE BLAS and WISEWAN, 2020.

Component and nutrient (g/kg)	Range
Protein	120-160
Crude fiber	140-200
Acid detergent fiber	170-n/a
Starch	0-140

2.4. Age and digestive problems

The conditions within the digestive system of rabbits changes significantly from the suckling stage to weaning and post-weaning. Rabbits tend to be most susceptible to pathogens and digestive disorders during the suckling-weaning stages, where their digestive system is already undergoing significant changes due to the transition of diet. In suckling rabbits, the stomach pH is around 5-6.5. In adults, the pH is 1-2. The high stomach pH of suckling rabbits allows for beneficial bacteria to pass the stomach and permits colonization of the hindgut, but also permits the passage of potentially pathogenic bacteria. By ingesting cecotropes from the dam, weanlings further the development of a healthy microbiota in their cecum. For this reason, early weaning can have significant detrimental effects. Once rabbits are weaned onto a high-fiber diet, the pH of the cecum decreases with the increased production of volatile fatty acids. The volatile fatty acids in turn influence the development of the microflora. Providing rabbits with a diet high in indigestible fiber is critical for preventing a disbalance of conditions in the gut during the weaning period (DE BLAS and WISEWAN, 2020).

2.5. Microflora and diseases

The cecum of the rabbit contains a diverse microflora that receives a steady supply of substrate from the small intestine. Variations to the makeup and quantity of ingesta reaching the cecum can result in a change to the microfloral balance. As mentioned previously, the microflora of the cecum consists primarily of the genera *Bacteroides, Bifidobacterium, Clostridium, Streptococcus* and *Enterobacter* (FANN and O'ROURKE, 2001). Microbes that may have potentially pathogenic consequences, such as *Clostridium* spp. can also be present in small quantities and may overgrow in their population if the microfloral balance is disrupted. During different pathologies, such as mucoid enteropathy for example, there may be a drop in large anaerobic metachromatic bacteria and protozoa, which are in high numbers in healthy rabbits. In addition, in mucoid enteropathy an increased number of coliforms has been demonstrated. The cecal microflora produces volatile fatty acids such as acetate and butyrate, and the latter has an important function in regulating the pH to remain between 5.7 and 6.1. A significant alteration to the pH of the cecum can result in overgrowth of pathogenic bacteria. Acetate is the predominant volatile fatty acid produced in healthy rabbits, and the amount of volatile fatty acids produced by the microflora have an impact on peristalsis. In a low fiber diet, less acetates are produced in comparison to butyrates and propionates. An abnormally high concentration of butyrates causes decreased gut motility. In rabbits that consume a steady high-fiber diet with relatively low changes, a resilient microflora forms that is relatively insensitive to small changes to the diet such as feeding of new foods. Contrastingly, particularly during the intensive production of rabbit meat where rabbits are kept in small enclosures and fed unnatural artificial diets, the intestinal microflora is much more sensitive to changes, and may allow the proliferation of pathogenic organisms. This is why proliferation of pathogenic bacteria is much more common in the gut of intensively kept rabbits (DONNELLY, 2003).

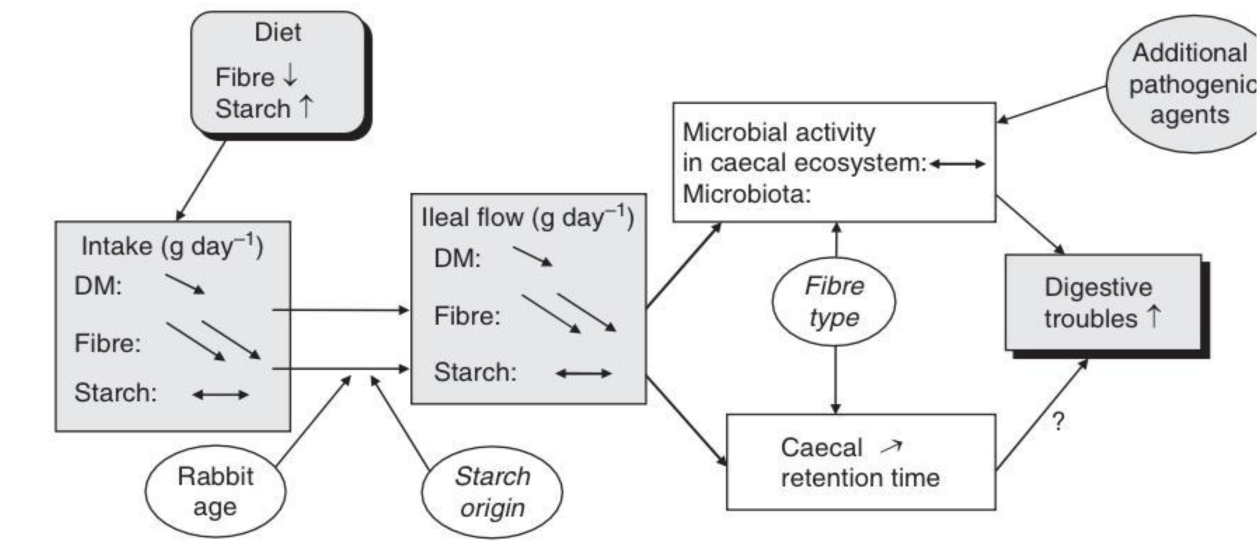

Figure 3. A flowchart demonstrating the relationship between a low-fiber diet and the incidence of digestive troubles in rabbits.

2.6. Trichobezoars and GI motility

Trichobezoars are compact pellets composed of primarily hair fibers consumed during regular grooming activities. While previously they have been considered to be a cause of anorexia and constipation, recent research shows that they are more likely to be a result of anorexia and a fiber-inadequate diet. Anorexia and low-fiber diets reduce gastrointestinal motility and increase retention time, facilitating the formation of trichobezoars. Significant stress can also result in trichobezoar formation. Clinical signs associated with trichobezoars include anorexia, depression, diarrhea, constipation and the presence of a firm mass in the epigastrium. It is important to emphasize that, as stated before, recent studies suggest that though these clinical signs are often associated with trichobezoars, they are not necessarily caused by them. Trichobezoars can be reliably diagnosed by endoscopy or other methods such as contrast radiography. Medical and fluid therapy is usually the primary treatment, except in cases of significant obstruction where surgery is necessary. Larger trichobezoars can cause complete obstruction of the intestinal tract and can result in severe complications such as necrosis or rupture of the digestive tract (OGLESBEE and LORD, 2020).

Figure 4. Blue arrow shows a trichobezoar that resulted in a gastric rupture (OGLESBEE and LORD, 2020).

A study in 1984 investigated the impact of trichobezoars on anorexia and fecal output in rabbits. Latex bezoars were applied to 12 rabbits to stimulate a trichobezoar, and they were monitored over the course of 24 weeks. No significant reduction in appetite nor fecal output was found, providing further evidence that trichobezoars are likely to be the result of anorexia, rather than the cause.

Another study described treating lack of peristalsis in rabbits with lack of appetite, reduced water intake, weight loss and reduced fecal output. The rabbits were treated with motility stimulants, fluids, and high-fiber feed (hay), and an improvement was seen. This study further highlights the likelihood that trichobezoars are a result of poor gut motility and increased intestinal retention time. Providing rabbits with a high-fiber feed such as hay has consistently been proven to reduce the risk of gut stasis and trichobezoar development (DONNELLY, 2003).

2.7. Clostridial enterotoxemia

Enterotoxemia in rabbits is a result of iota-like toxin secretion by bacterium *Clostridium spiroforme*. Other *Clostridium* species may be present simultaneously but are not considered to be causative agents of enterotoxemia. *Clostridium spiroforme* is not part of the normal microflora of healthy rabbits and can proliferate especially quickly in those rabbits with an undeveloped microflora, such as newly weaned rabbits. Adults may also be affected, but a predisposing factor that disrupts the microbial floral balance is required, such as stress, a radical change in diet or concurrent diseases (JENKINS, 2004). Proliferation of *Clostridium spiroforme* causes significant cecal dysbiosis. Enterotoxemia may occur in rabbits in acute or chronic form. The acute form of the disease is characterized by anorexia, depression, watery and sometimes bloody diarrhea and perineal soiling. In the chronic form, rabbits are anorexic, and exhibit less severe diarrhea and weight loss. Upon necropsy, hemorrhages, mucus and gas can be found throughout the entire digestive tract (MAINIL, 2006). Enterotoxemia was first identified as a disease in 1936, although the etiological role of *Clostridium* was not demonstrated until 1982 by Carman and Borriello. As much as 52% of rabbits exhibiting digestive pathologies such as diarrhea have been shown to be positive for *Clostridium spiroforme*. Although rabbits of all ages may be infected by *Clostridium spiroforme*, younger, newly weaned rabbits are more commonly affected due to their less developed and more sensitive microbiota. Affected rabbits exhibit clinical signs such as weight loss, diarrhea, and depression, or may be found dead without obvious preceding signs. Upon necropsy, copious watery cecal contents may be found along with hemorrhagic typhlitis and mucous contents in the colon. A study in 1985 studied cecal samples from commercial rabbits exhibiting signs of enteritis. It suggested that a changed microfloral balance, as a result of a poor diet, predisposed rabbits to *Clostridial* colonization and proliferation, and the resulting iota-like toxin production. They also found that *Clostridium spiroforme* was rarely present in healthy rabbits, although it is a relatively common finding in commercial rabbitries (PEETERS et al., 1986). This hypothesis is further supported by findings which concluded that *Clostridium spiroforme* was not a normal component of the microflora of a healthy rabbit, and that the microfloral balance must be disturbed in order for *Clostridium spiroforme* to proliferate and cause clinical disease (CARMAN and BORRIELLO, 1984).

2.8. Epizootic rabbit enteropathy

Epizootic rabbit enteropathy is a disease of rabbits associated with an expansion of the *Clostridium* taxon within their gut microflora, particularly *Clostridium cuniculi*, though no specific etiological agent has been confidently confirmed. This proliferation of *C.cuniculi* is thought to be a result of an infection by the bacterium, rather than proliferation of an opportunistic pathogen already present in the microflora. It is thought to be transmissible through the intestinal contents of ill animals and enters exposed animals orally. ERE is frequently associated with high mortality rates of up to 95% and can thus lead to devastating economic consequences in the commercial rabbit industry.

In a study at the Polytechnic University of Valencia, 33 rabbits were housed individually after weaning with ad libitum food and water and followed to investigate ERE development. The only difference in the microbiota of the rabbits that developed ERE was a significant expansion of the *Clostridium* taxon, and this expansion was considered the only link to the initiation of disease (DJUKOVIC et al., 2018).

The link between high fiber diets and ERE development and treatment has been investigated, but further research is required to confirm any significant effect. Rabbits with ERE treated with high fiber diets in a 2022 study maintained a smaller body weight and different microfloral makeup than rabbits unaffected by ERE. However, the incidence of diarrhea and excess mucus production in the intestine was reduced in the rabbits fed with a high fiber diet. A neutral detergent fiber concentration of at least 36% showed some benefits in regards to treatment of ERE (PUÓN-PELÁEZ et al., 2022).

2.9. Tyzzer's disease

Tyzzer's disease is a bacterial disease caused by an obligate intracellular gram-variable rod-shaped *Clostridium piliforme,* which produces resistant spores excreted in feces. Weanling rabbits are most often affected, and various factors such as inadequate husbandry, overcrowding, a low-fiber diet and presence of other diseases may predispose rabbits to the disease. Rabbits are infected by oral ingestion of spores from feces. The bacterium invades the epithelium of the small intestine and cecum and may persist in a latent state without causing disease. Clinical disease is triggered by immunosuppression. The pathogenesis of Tyzzer's disease involves cecal, intestinal, liver and heart necrosis, though intestinal lesions may appear more peracutely while lesions in the heart occur in the later stages of disease (DONNELLY, 2003).

Figure 5. Necrotic and hemorrhagic intestinal lesions. Source: Department of Natural Resources; www.michigan.gov, accessed 25 March 2024.

The disease can exist either in an acute or, less commonly, chronic form. The acute form of the disease is characterized by diarrhea, significant loss of condition and often death within 1-2 days. Younger, weanling rabbits are most commonly affected by the acute form (ARTUKOVIĆ et al., 2010). In chronic disease, rabbits suffer less severe diarrhea and loss of condition. Older rabbits are more likely to exhibit the chronic form of the disease. If treatment is postponed to a later stage of disease, it is often unsuccessful. Early treatment of exposed animals with a high-fiber diet and fluids has been proven effective in preventing severe disease. Appropriate husbandry and hygiene is also critical, as the resistant spores require at least a 0.3% sodium hypochlorite solution or temperatures as high as 80ºC to be killed (OGLESBEE and LORD, 2020).

Though members of the *Clostridium* genera are a part of the normal microflora of the rabbit, *Clostridium piliforme* is considered an infectious pathogen not normally present in the healthy rabbits. As in other *Clostridium* species, its ability to proliferate successfully in the gut may rely to some extent on an imbalance of the microbiota. This imbalance could be a result of an improper diet such as one too high in starch and too low in fiber, resulting in abnormal volatile fatty acid production and cecal pH alteration.

2.10. Protective effects of fiber and clostridium

Indigestible fiber has been shown to provide a protective effect in rabbits against the proliferation of potential pathogenic bacteria such as ***Clostridium*,** which can cause diseases such as enterotoxemia and Tyzzer's disease (ZHENG et al., 2018). A diet low in indigestible fibers can alter pH levels in the cecum through excessive butyrate production, or lower peristalsis through reduced acetate production, leading to a change in the microflora and a reduced gut motility. This allows potentially pathogenic bacteria such as *Clostridium* and coliforms to remain in the gut longer and to proliferate in the absence of a healthy balanced microflora, as the beneficial bacteria struggle to survive within the altered pH environment. A diet low in indigestible fiber can also result in indirect consequences, as the concentration of available carbohydrates would be higher in such a diet, resulting in carbohydrate overload that supports the proliferation of potential pathogens.

2.11. Colibacillosis

As mentioned previously, the rabbits' cecal pH can be altered by a low-fiber diet. This predisposes rabbits to various infectious pathogens, including colibacillosis. Colibacillosis is enteritis caused by proliferation of bacterium *Escherichia coli* in the intestine, and is often found with other concurrent intestinal pathologies such as coccidiosis (MCWILLIAMS, 2001).

There are four groups of pathogenic strains of *E. coli*: enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli* and enterohemorrhagic *E. coli*. In rabbits, enteropathogenic *E. coli* is most relevant, and is often referred to as rabbit diarrhea *E. coli* (RDEC-1). Colibacillosis occurs most frequently in newly weaned rabbits, and clinical signs include watery yellow diarrhea, dehydration, weight loss and soiling of the perineum. Upon necropsy, hemorrhagic lesions may be found in the cecum walls (MAERTENS and COUDERT, 2006).

Young, newly weaned rabbits are most susceptible to colibacillosis. The rabbits exhibit clinical signs typical for enteropathies such as diarrhea and dehydration. Depending on the virulence of the particular strain, rabbits may die acutely or survive and exhibit poor growth. Upon necropsy, copious liquid contents and gas may be found within the small intestine and cecum. The walls of the cecum are thin with scattered hemorrhages (COUSSEMENT et al., 1984).

Due to the possibility of residues in food and the difficult nature of bacterial enteric infections, the use of antibiotics for treatment of colibacillosis should be avoided. Instead, particularly within the European Union, breeders are relying on alternative methods such as diet and preventive husbandry and hygiene to manage colibacillosis. Though there is a lack of research regarding treatment and prevention of colibacillosis with high fiber diets, the protective effect of fiber and a healthy microflora cannot be overlooked.

2.12. Mucoid enteropathy

Mucoid enteropathy is a syndrome often affecting younger rabbits aged from as young as 3 weeks up to 14 weeks old. Mucoid enteropathy is characterized by a loss of appetite, diarrhea, depression, an enlarged abdomen, condition loss and increased mucus excretion into the intestine by goblet cells. A classic sign of mucoid enteropathy is bruxism, resulting from abdominal pain. The exact cause of mucoid enteropathy is unknown, and thus mucoid enteropathy can be referred to by additional terms, such as mucoid enteritis or mucoid enteritis complex. Recent studies show increasing evidence that a low-fiber diet creates the necessary predisposing changes to cause this syndrome. These changes include a reduced gut motility, a changed cecal pH due to abnormal production of volatile fatty acids, and a disrupted microfloral balance (OGLESBEE and LORD, 2020). Mucoid enteropathy has been reported in both commercial production as well as in pet rabbits, though the latter is very rare. A specific causative agent has not been identified, though predisposing factors such as a low-fiber diet have been suspected to play a significant role.

While studies into mucoid enteropathy often involve taking samples from the cecum for bacteriological examination, it has not been confirmed that an infectious pathogen is the cause of this disease. A study in 2000 at the University of Valencia in Spain covered the first case of naturally occurring mucoid enteropathy in a specific pathogen free rabbit. The 7-month-old doe was euthanized for necropsy, and the contents of the cecum were sampled for bacteriological examination. No likely pathogens were identified, and due to the SPF-nature of the doe's production, it can be assumed that contaminating pathogens did not play a role in disease development (VANDEKERCHOVE et al., 2000).

In a study in Turkey at the Faculty of Veterinary medicine and the Medicine faculty, 20 rabbits between the ages of 3-7 weeks old were investigated. The rabbits had diarrhea and enlarged abdomens, and exhibited a relatively high mortality rate, around 15-35%. Upon necropsy, an enlarged stomach and jejunum were found, with large amounts of liquid contents and gas. The colon was found to be distended as well, with a significant accumulation of mucus. Histologically, goblet cell hyperplasia was found within the small intestine mucosa. The rabbits had been fed a fiber-inadequate diet, and the surviving rabbits were treated with a low-energy, high-fiber diet. Low fiber causes increased retention time, while an increased starch concentration results in a changed ratio of volatile fatty acids in the cecum and increased lactate production. It was suspected that the primary culprit for the pathological changes may have been a poorly formulated diet inadequate in fiber and too high in starch (HALIGUR et al., 2009).

Mucoid enteropathy is a significant cause of economic losses in commercial rabbit production, with mortality rates sometimes as high as 60% (HU et al., 2018). As mentioned before, the etiopathogenesis of mucoid enteropathy is not well established. While specific risk factors have not been proven, studies suggest that it is likely that factors such as diet, stress, concurrent diseases and dysbiosis play a role in ME development. Furthermore, research into the etiopathogenesis of mucoid enteropathy is often disrupted by the development of infections such as coccidiosis, *Clostridial* overgrowth or *Colibacillosis*. None of these infectious agents have yet been implicated for the development of mucoid enteropathy. The clinical presentation of mucoid enteropathy can also be misleading, as rabbits with the disease can exhibit less typical signs such as respiratory distress, due to increased mucus production within the respiratory tract. Mucoid enteropathy is primarily a disease of intensive commercial rabbitries. In commercial rabbitries veterinary treatment is scarcely sought out, and home treatment or culling is preferred. In the rare cases of mucoid enteropathy in pet rabbits, prognosis is extremely poor as very young rabbits are most often affected, and euthanasia is the likely outcome. While there is a lack of research proving the effectiveness of antibiotics for mucoid enteropathy treatment, they are still commonly used by breeders. This should be generally discouraged, as there is a lack of information regarding the cause of mucoid enteropathy, and unregulated use of antibiotics can lead to severe consequences in the realm of antimicrobial resistance (DONNELLY, 2003).

3. CONCLUSION

The digestive system of the rabbit, particularly during sensitive periods such as the weaning period, can be susceptible to a variety of digestive disorders with varying mortalities. The homeostasis and proper functioning of the digestive tract relies critically upon an adequate concentration of nondigestible fiber within the diet, as well as a sufficiently low concentration of starch in comparison to fiber. Thus, a proper fiber-to-starch ratio is important in the maintenance of proper functioning of the digestive tract. A diet sufficient in indigestible fiber keeps a proper ratio of volatile fatty acids in the cecum, as a low-fiber diet would increase butyrate production and result in lower gut motility. Low-fiber diets also disrupt the cecal pH and cause reduced appetite in rabbits. During sensitive periods such as the weaning period, the rabbits' microflora is undergoing significant development and is not yet the completely balanced microflora that can be found in healthy adults. The cecum is particularly susceptible to colonization and overgrowth by infectious or opportunistic bacteria like *Clostridia* or coliforms. The most common clinical signs of digestive upset in rabbits include diarrhea, anorexia, and a distended abdomen. Unfortunately, these clinical signs are rather nonspecific, as are often the findings during necropsy. It can be difficult to identify a specific etiological agent as the cause of a digestive disease, as many bacteria can be normal components of the rabbit microflora, like members of the *Clostridium* taxon. Additionally, because these digestive disorders often occur due to similar predisposing conditions and during the weaning period, many different types of bacteria can be found during bacteriological examination, and it can be troublesome to separate the disorders from one another and identify a primary disease. For many digestive diseases such as mucoid enteropathy, the etiological agent has not yet been identified. This causes further problems for treatment and prevention of the disease, though fortunately a link has been established with fiber, and the efficacy of high-fiber diets with preventing and treating mucoid enteropathy. Across all the digestive diseases discussed, maintenance of a high-fiber diet is a critical factor in prevention. With rabbits being hindgut fermenters and herbivores, an adequate concentration of indigestible fiber ensures the proper conditions for their digestive tract to function properly. While many factors besides fiber play a role in digestive disease development, the role of fiber should not be overlooked in rabbits.

4. REFERENCES

1. SOHN, J., M. A. COUTO (2012): Anatomy, physiology, and behavior. In The Lab. Rab. Guin. Hams. Oth. Rod.; pp. 195-215.
2. DE BLAS, C., J. WISEWAN (2020): Nutrition of the Rabbit, pp. 19-229.
3. KAY, R. (1982): Dietary fiber. Journ. Lip. Res. 23, 221-242.
4. FANN, M. K., D. O'ROURKE (2001): Normal bacterial flora of the rabbit gastrointestinal tract: a clinical approach. Sem. Avi. Exot. Pet Med.; pp. 45-47.
5. DONNELLY, T. M. (2003): Textbook of rabbit medicine. 1st edition, Butterworth-Heinemann Publishing, Oxford, pp. 252-254.
6. OGLESBEE, B. L., B. LORD (2020): Gastrointestinal diseases of rabbits. Ferr. Rab. Rod. 174-187.
7. JENKINS, J. R. (2004): Gastrointestinal diseases. Ferr. Rab. Rod. 161-171.
8. MAINIL, J. (2006): Genus Clostridium-Clostridia in medical, veterinary and food microbiology: Diag. Typ. 88-90.
9. PEETERS, J., R. GEEROMS, R. CARMAN, T. WILKINS (1986): Significance of Clostridium spiroforme in the enteritis-complex of commercial rabbits. Vet. Microbiol. 12, 25-31.
10. CARMAN, R. J., S. P. BORRIELLO (1984): Infectious nature of Clostridium spiroforme-mediated rabbit enterotoxaemia. Vet. Microbiol. 9, 497-502.
11. DJUKOVIC, A., M. GARCIA-GARCERA, E. MARTÍNEZ-PAREDES, S. ISAAC, A. ARTACHO, J. MARTÍNEZ, C. UBEDA (2018): Gut colonization by a novel Clostridium species is associated with the onset of epizootic rabbit enteropathy. Vet. Res. 49, 1-14.
12. PUÓN-PELÁEZ, X.-H. D., N. R. MCEWAN, R. C. ÁLVAREZ-MARTÍNEZ, G. MARISCAL-LANDÍN, G. M. NAVA-MORALES, J. MOSQUEDA, A. M. OLVERA-RAMÍREZ (2022): Effect of feeding insoluble fiber on the microbiota and metabolites of the caecum and feces of rabbits recovering from epizootic rabbit enteropathy relative to non-infected rabbits. Pathog. 11, 571.

13. ARTUKOVIĆ, B., Ž. GRABAREVIĆ, A. G. KURILJ, A. BECK, K. SHIROTA, Y. SAKURADA, S. KAWAMURA, P. DŽAJA (2010): Clinical and pathological findings of an outbreak of Tyzzer's disease in a rabbit colony in Croatia. 80 (6), 761-770.
14. ZHENG, W., K. WANG, Y. SUN, S.-M. KUO (2018): Dietary or supplemental fermentable fiber intake reduces the presence of Clostridium XI in mouse intestinal microbiota: The importance of higher fecal bacterial load and density. PloS one. 13, e0205055.
15. MCWILLIAMS, D. A. (2001): Nutritional pathology in rabbits: Current and future perspectives. In Proceedings of the Prepared and Presented for the Ontario Commercial Rab-bit Growers Association (OCRGA), 20th October, Ontario, Canada, pp. 5.
16. MAERTENS, L., P. COUDERT (2006): Recent advances in rabbit sciences, Institute for Agricultural and Fisheries Research, Belgium, pp. 171-180.
17. COUSSEMENT, W., R. DUCATELLE, G. CHARLIER, L. OKERMAN, J. HOORENS (1984): Pathology of experimental colibacillosis in rabbits. Zentr. Vet. Reih. B. 31, 64-72.
18. VANDEKERCHOVE, D., G. CHARLIER, S. ROELS (2000): A naturally occurring case of mucoid enteropathy in a specific pathogen free (SPF) rabbit. In Proceedings of the Proceedings of the 7th World Rabbit Congress, 4-7th July, Valencia, Spain, pp. 363-368.
19. HALIGUR, M., O. OZMEN, N. DEMIR (2009): Pathological and ultrastructural studies on mucoid enteropathy in New Zealand rabbits. Journ. Exot. Pet Med. 18, 224-228.
20. HU, B., Z. FAN, H. WEI, Y. SONG, R. QIU, M. CHEN, W. XU, J. XUE, F. WANG (2018): Detection of mucoid enteropathy syndrome disease in rabbit farms in East China. Res. Vet. Sci. 119, 259-261.

5. SAŽETAK

Uloga ugljikohidrata hraneu probavnim poremećajima kunića

Rebecca Reponen

Kunići su biljojedi s poslije želučanom fermentacijom koji se oslanjaju na odgovarajuću koncentraciju vlakana u svojoj prehrani kako bi održali zdravlje probavnog sustava. Unutar prehrane kunića, vlakna i škrob igraju ključnu ulogu u ravnoteži mikroflore i pH, kao i održavanju motiliteta crijeva. Kada se omjer škroba prema vlaknima poveća, više škroba postaje dostupno mikroorganizmima cekuma za fermentaciju. To mijenja koncentraciju nižih masnih kiselina, što rezultira promijenjenim pH i mogućom promjenom motiliteta. Smanjeni unos vlakana također rezultira produljenim vremenom zadržavanja sadržaja u probavnom sustavu. Ove promjene predisponiraju kuniće za razne probavne poremećaje, uključujući infekcije bakterijama roda Clostridia i nekim koliformima. Klostridije uzrokuje bolesti kao što su Tyzzerova bolest, epizootska enteropatija kunića i klostridijska enterotoksemija, koje karakteriziraju proljev, gubitak težine i iznenadna smrt. Vrste koje sudjeluju su: *Clostridium piliforme, Clostridium cuniculi i Clostridium spiroforme.* Iako postoje različiti zajednički predisponirajući čimbenici za ove bolesti, jedan od najvažnijih je nedostatak vlakana u prehrani kunića. Prehrana bogata vlaknima pokazala se preventivnom i korisnom u liječenju ovih bolesti, iako nedostaju podaci o njihovoj izravnoj povezanosti. Uzimajući u obzir štetne učinke prehrane s malo vlakana na o zdravlje probavnog sustava kunića, može se zaključiti da takva prehrana stvara uvjete potrebne za razmnožavanje oportunističkih i zaraznih bakterija i izazivanje bolesti. Za liječenje ovih bolesti koriste se antibiotici i stimulatori motiliteta crijeva, ali prevencija je ipak ključna, posebno u intenzivnoj komercijalnoj proizvodnji kunića gdje ekonomski gubici mogu biti značajni. Iz svega navedenog, jasni je da u prehrani kunića naglasak treba staviti na osiguravanje hrane bogate vlaknima, ali s relativno niskim sadržajem škroba. Sadržaj sirovih vlakana od 140-200 g/kg i najveći sadržaj

škroba od 140 g/kg preporučuje se za održavanje zdravog, funkcionalnog probavnog sustava trakta kunića.

Ključne riječi: poslije želučana fermentacija, ugljikohidrati, probavni poremećaji

6. ABSTRACT

Rebecca Reponen

The role of dietary carbohydrates in digestive disorders of rabbits

Rabbits are herbivore hindgut fermenters and rely on an adequate concentration of fiber within their diet to maintain digestive health. Within the diet of the rabbit, fiber and starch play a critical, interconnecting role regarding the balance of microflora and pH, as well as maintenance of gut motility. When the ratio of starch to fiber is increased, more starch is made available to the cecal microbiota for fermentation. This changes the concentration of volatile fatty acids, resulting in a changed pH and a possible alteration of motility. Reduced fiber also results in an increased retention time. This disruption of gut balance predisposes rabbits to a variety of digestive disorders, including infections by bacteria of the taxon *Clostridia* and some Coliforms. *Clostridia* causes diseases such as Tyzzer's disease, epizootic rabbit enteropathy and *Clostridial* enterotoxemia, which are characterized by diarrhea, weight loss and sudden death. *Clostridial* species *Clostridium piliforme, Clostridium cuniculi* and *Clostridium spiroforme* have been implicated. One of the most important predisposing factors may be the lack of fiber in the rabbits' diet. A high fiber diet has been shown to be preventative and beneficial in treatment of these diseases, although there is a lack of data regarding their direct connection. Considering the detrimental effects a low-fiber diet has on the digestive health of the rabbit, it can be concluded that such a diet creates the conditions necessary for opportunistic and infectious bacteria to proliferate and cause disease. While antibiotics and motility promoters have been used for treatment of digestive upset in rabbits, prevention is key, especially in intensive commercial rabbit production where economical losses from mortalities can be significant. An emphasis should be put on providing rabbits with a high-fiber and a comparatively lower starch content diet. A crude fiber content of 140-200g/kg and a maximum starch content of 140g/kg is recommended to maintain a healthy, functioning digestive tract.

Keywords: Hindgut fermenter, diet, fiber, *Clostridium*

7. CURRICULUM VITAE

I was born December 8th, 1998 in Helsinki, Finland. In 2011 I moved to Hong Kong with my family, and attended Discovery College from 2012 until 2017, obtaining my International Baccalaureate diploma in the process. During my International Baccalaureate studies, I studied Biology, Psychology and English language and literature at higher level, and Math studies, Arts and Finnish language A at standard level.

In 2015 in Tung Chung, I volunteered at an SPCA animal rescue center for 4 weeks, to walk the dogs and aid in their socialization and daily exercise needs. In 2019 in Discovery Bay, I completed a week-long internship at Island Veterinary Services, where I acted as an assistant and aided the veterinarians and nurses during dairy activities. From 2018 onwards I have attended the Faculty of Veterinary Medicine at the University of Zagreb in Croatia, in their English program for veterinary medicine. Intermittently before and during the 2020 COVID pandemic I acted as an assistant during histology classes at the faculty and sent materials for classes when classes were conducted electronically from home. In Summer of 2023 I completed a 2-week long surgical birth control and anesthesia training course in Chiang Mai, Thailand through World Veterinary Service. My longest internship was for 6 weeks in Helsinki at Mevet Veterinary Hospital in 2024, where I acted as a clinical assistant and veterinary nurse.