University of Veterinary Medicine Budapest
Department of Pathology

THESIS

Epizootic rabbit enteropathy: History, effects on the rabbit industry and recent advances in molecular microbiology

By Chiara Gisela Monika Feige

Supervisor: Dr. Német Zoltán László PhD

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Abbreviations

ERE	Epizootic rabbit enteropathy
SPF	Specific pathogen free
ME	Mucoid enteritis
AL	Ad libitum
RES	Restricted group (in terms of % AL feed intake)
DWG	Daily weight gain
FCR	Food conversion rate
IU	International units
PPM	Parts per million
PCR	Polymerase chain reaction
NDF	Insoluble fibre

Abstract

Epizootic Rabbit Enteropathy (ERE) is a complex gastrointestinal disease affecting domestic rabbits, characterized by abdominal distention, acute enteric distress, and increased morbidity and mortality. It is the primary cause of mortality in the European rabbit industry and costs millions of Euros every year. It is known to be potentially fatal to different rabbit ages and persists in threatening the rabbit production industry. It is mostly documented in intensive and semi-intensive fattening farms, but there are also reports of ERE in pet rabbits, which are rather rarely seen. It is known to be a contagious disease with a 30-40 % mortality and even up to 100% morbidity in up to a few days. Clinical signs appear one day after infection and the disease reaches its peak four to six days later. The disease is reproduced by the inoculation of intestinal contents from infected rabbits. Predisposing factors may include a low-fibre diet, poor husbandry and hygienic conditions, and the duration of the weaning period of rabbit kits. Up until this day, the causative aetiology of ERE is still uncertain and can only be narrowed down to a certain number of agents involved, but there is no final identification of the actual agent yet.

This thesis provides a comprehensive exploration of ERE, containing its historical background, influences on the rabbit industry, and contemporary advances in microbiological research.

1 Introduction

Epizootic rabbit enteropathy (ERE) is a condition threatening the meat rabbit industry all over the world [1]. The disease quickly moved through rabbit farming areas internationally and has resulted in significant losses in the rabbit farming sector [2, 3]. It is known to be a contagious disease among mostly meat-producing rabbits with a 30-40% mortality and up to 100% morbidity in up to a few days [4]. Without the use of medication, mortality rates can reach up to 80% in the acute phase of the disease [5]. Clinical signs appear one day after infection and the disease reaches its peak four to six days later [4]. These acutely occurring clinical signs include rambling noises and a distended abdomen. Additionally, we can observe a significant decrease in growth from the second day following mucous excretion, small amounts of watery diarrhoea and caecal impaction [4, 6]. The aetiology or pathogenesis of this disease is not defined yet, however, it has been confirmed that it can be linked to nutritional, environmental and microbial risk factors [7].

This thesis focuses on the historical evolution of the disease as well as on the ongoing research to define its causative agents. It is divided into main parts such as the historical perspective, the aetiology, and pathogenesis of the disease, and the management and treatment technologies followed by the most recent advancements in microbiology.

ERE displays its importance in its impact on the rabbit industry. It is the first cause of mortality in the European rabbit industry and costs approximately ten million Euros yearly in Belgium [8]. Its appearance in 32% of all enteropathies troubling Mexican rabbit farms represents its worldwide challenging occurrence [7]. As mentioned before, it can cause up to 100% morbidity and 30-40% mortality [4] which is a significant economic loss for rabbit farmers. Mortality and morbidity are always caused by the named acute digestive symptoms [9]. Even though ERE is not always fatal, it shows that survivors have a lower weight gain compared to healthy rabbits in the same production system which results in a lower quantity of meat being produced. Furthermore, we can observe as much as 25% decrease in fertility of female rabbits and up to 15% decrease in libido in affected males. Consequently, a smaller number of kits per cycle will be produced [1].

2 Historical perspective

ERE was originally called mucoid enteropathy and more recently mucoid enteritis. This symptomatic was firstly mentioned over 100 years back with symptoms very similar to what we see today, however, it was not called enteropathy at that time. Mucoid enteropathy has been described for over 40 years. ERE, as we know it today, was first documented in both France in late 1996 and in Galicia in Spain in September 1996. In the case of Galicia, at least 700 farms were affected by the rapid spread by the end of 1997. During monitoring of French farms every six months from 1996-2002, it turned out that more than 90% of French rabbit farms were either acutely or latently affected by ERE [1, 6].

The disease then rapidly spread to other parts of Europe like Portugal, Belgium, The Netherlands, Hungary, and the United Kingdom [4]. Even though it was first reported in Europe, ERE has become an international problem which means this disease is not endemic in Europe anymore, we can identify it as a pandemic [1].
The first outbreak was characterized by abdominal distention and the onset of sudden diarrhoea in 6–14-week-old rabbits. Macroscopical lesions included gaseous dilation of the stomach and a mostly liquid-filled small intestine [10]. Because of the appearance of symptoms right after a delivery of commercial feedstuffs on a rabbit farm, the feed was originally suspected to be carrying the infectious agent, but this hypothesis has been eliminated through various tests and studies. However, it was later discovered that feed could be a passive vector of the disease if the feed is taken from a feeder in an infected breeding establishment, but its virulence does not persist longer than 3 to 4 months [4, 6].

Since its first occurrence in 1997, research has progressed to determine the aetiological agent of ERE. Unfortunately, to this day we were only able to narrow it down to a certain number of agents that are involved, but there is no clear definition of the actual agent yet [11].

Mortality caused by ERE began to decrease after strict hygienic measures were taken and active treatment with antimicrobials like Bacitracin and Tiamulin was used [1, 6].
The next objective in diagnostic research included finding an inoculum that reproduced the disease successfully. Using specific pathogen-free (SPF) rabbits, the disease was reproduced by inoculating intestinal contents from either diseased or dead animals into healthy SPF rabbits.

Considering this, other factors like feeds, toxins, or pesticides could be eliminated as potential exclusive causative agents. Further studies in connection to the way of inoculation itself revealed that oral inoculation (via the drinking water or administered via aerosol on feedstuffs or directly onto the nose) was more effectively reproducing the disease than inoculation of the intestinal contents directly into the stomach via an oesophageal catheter. The underlying cause for this may be that the pathogen is involved in a primary cycle that acts in the upper gastrointestinal tract. ERE was also experimentally reproduced from contaminated foodstuffs, direct contact between individuals, and contact with infected breeding materials such as feeders, cages, and drinkers if they were not disinfected but only washed [6]. During different study trials, it was obvious that different groups of rabbits kept in the same room during the study would re-infect each other most likely due to recontamination through the untreated groups [12].

Healthy SPF rabbits were inoculated with the caecal contents of diseased rabbits to reproduce the symptoms [10]. These so-called TEC-inocula were obtained by mixing the caecal contents of SPF rabbits that were previously infected with ERE [13]. The first generation of TEC was TEC1 and the last is TEC4 [8].

These intestinal contents contain a large number of different microorganisms. To reduce the number of microorganisms in the inoculum, the French reference inoculum TEC3 was fractionated on a discontinuous sucrose gradient which resulted in 7 different fractions: supernatant, 10%, 20%, 30%, 40%, 50%, and pellet. Specific pathogen-free rabbits were then inoculated with three of those fractions (supernatant, 30%, and pellet). The study was obtained to define the bacteriological agents in all 7 different fractions, to confirm whether the aetiological agent(s) were present in the fraction by inoculating rabbits, and to single out a fraction that can replace the reference inoculum TEC3 in certain cell cultured or egg inoculation since the TEC3 inoculum has proven to be unsuitable for applications such as cell cultures and egg inoculation [11].

It turned out that the agent was present in at least 3 of the inocula and it does not seem to be a bacterial species we are able to cultivate on our known bacterial culture media [11].

3 Impact on the rabbit industry and risk factors

ERE is a highly prevalent and economically significant disease affecting the rabbit industry worldwide. ERE impacts commercial rabbit farming and rabbit breeders, resulting in considerable financial losses caused by reduced productivity, increased mortality, treatment costs, and impacts on reproduction [1].

Furthermore, gastrointestinal problems lead to substantial health issues, including reduced growth and inefficient feed utilization. These issues frequently result in more significant financial losses than mortality [14]. ERE's first clinical sign to be observed is a decreased feed intake [5]. Subsequently, this leads to a decreased productivity in meat-producing rabbits and therefore an economic loss for the farmers.

The treatment of ERE with antibiotics carries the risk of acquiring multi-resistant bacteria which may result in a proportion of healthy animals being permanent carriers of not only ERE but also other digestive pathologies [14].

A retrospective case study was performed by LE BOUQUIN ET AL (2009) to determine specific risk factors which predispose young rabbits to ERE. Certain rabbit farms in western France contributed to the study as either a "case" farm or a "control" farm. "Control" farms were farms in which no clinical lesions of ERE were observed and the overall fattening rabbit mortality in the last 5 batches was less than 10%. The average mortality was 19.5% in "case" farms and 5.6% in "control" farms. The likelihood of ERE occurrence was higher when rabbits were weaned later, specifically after 35 days, compared to earlier weaning. This likelihood increased when young weaned rabbits were moved to a specific fattening room instead of transferring both, the does and the young rabbits to the room where they were born. Additionally, the risk of ERE after weaning was higher when the volume of the fattening room exceeded 0.14 m^3/kg and was strongly connected to a high mortality rate of at least 10.5% in young rabbits before weaning [5].

Keeping these risk factors in mind, the author has come up with several hypotheses to avoid or decrease the possibility of infection. It is suggested that weaning rabbits at an earlier stage may decrease the transmission of pathogens, which has been observed in the case of *Pasteurella multocida*. Moreover, early weaning involves providing specialized nutrition to young rabbits sooner, facilitating their adjustment to solid food intake at an earlier stage around weaning time.

The approach used for moving animals during weaning appears to be relevant in managing the risk of infection. The research highlights the beneficial impact of transferring the mothers during weaning instead of the young rabbits. This approach can prevent the mixing of animals of different ages in the fattening room. Moving the does reduces the stress experienced by the young rabbits due to handling and, importantly, the differences in temperature and humidity between the birthing and fattening rooms. Additionally, this practice enables the routine cleaning of the kindling room. The density of rabbits in the fattening room was lower in "case" farms compared to "control" farms. This may indicate challenges in maintaining ideal atmospheric conditions in the affected farms, potentially due to less effective ventilation. Many rabbit farms have transferred from small-scale operations to larger, intensive production institutions, and sometimes, the existing buildings have been adapted with insufficient consideration for environmental conditions. Nevertheless, these environmental conditions are critical for ensuring optimal technical performance [5].

GUITIAN ET AL. (2000) assert that the environment and its microbial content play a significant role in the occurrence and severity of ERE in commercial farms. Rabbits are especially sensitive to alterations in their environment, including changes in water pH, temperature, humidity, and the concentration of ammonia (NH_3) [5, 15].

4 Aetiology and pathogenesis

4.1 Clinical signs and lesions

The first detectable clinical sign of ERE is the decrease or loss of appetite. This is often followed by a distended abdomen, mild watery diarrhoea, occasional mucus excretion, and caecal impaction as seen in **Figure 1**. These signs are associated with sudden high mortality and are mostly seen in recently weaned fattening rabbits during the age of six to 14 weeks. It may be difficult to differentiate ERE from other enteropathies due to the ambiguity of the clinical signs. During necropsy of the gaseous, distended stomach and small intestine as seen in **Figure 2**, we find no significant macroscopic evidence that represents lesions typical for an inflammation, neither acute nor chronic. Clinical symptoms and apparent lesions of the disease show similarities with those of mucoid enteritis. However, mucoid enteritis cases that were reported were rather of sporadic nature in contrary to ERE which has an epidemic character [4–7, 16].

Macroscopic lesions appear to be more critical during the beginning of the monitoring period than in the end during different trials. Fluid accumulations in the upper gastrointestinal tract are most probably caused by secretory diarrhoea due to an absence of an inflammatory response. Furthermore, bacterial enterotoxins are likely to take part in an active chlorine secretion and/or suppress NaCl absorption of the intestinal epithelial tissue [16]. Additionally, we can observe a decrease in the pH in the stomach, parts of the duodenum, and the urine as well as an increase in the pH in the colon. The increased pH in the colon is believed to be caused by microbial dysbiosis while the decreased pH in the stomach is explained by a lack of feed [7, 17, 18].

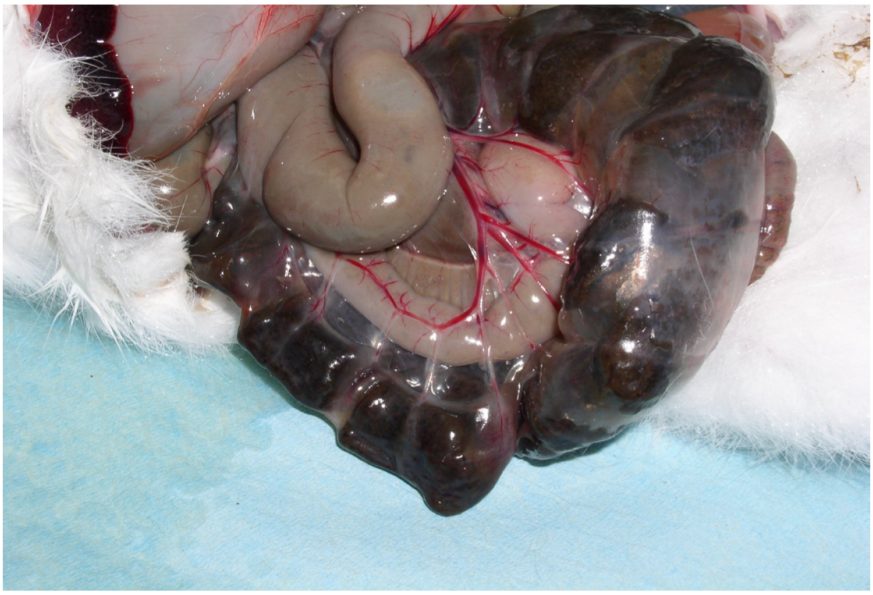

Figure 1: Caecal impaction of a six-week old rabbit with experimentally produced ERE [4].

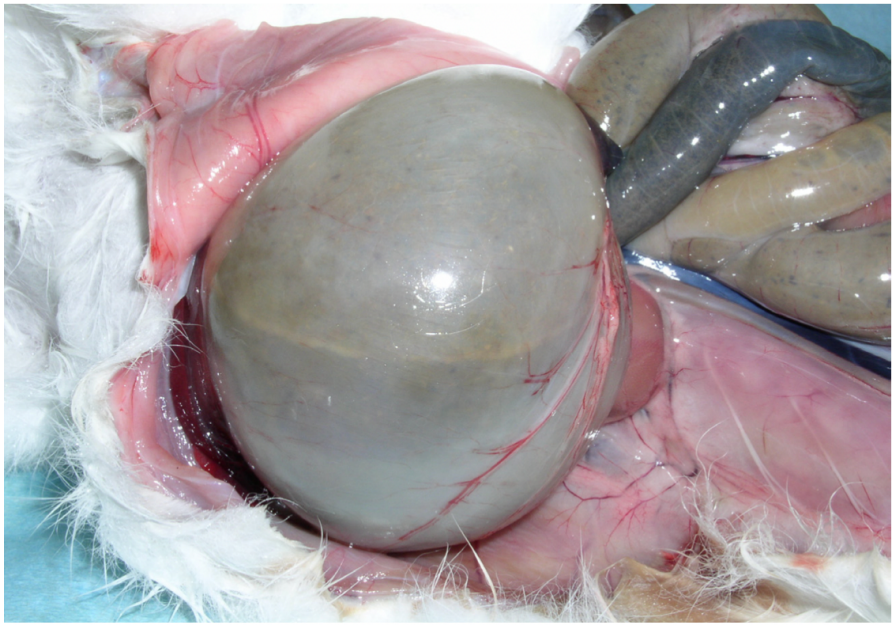

Figure 2: Distended and liquid filled stomach and small intestine in the absence of inflammatory lesions are considered typical lesions of ERE in a six-week old rabbit [4].

It is important to mention that also microscopic lesions reveal only limited inflammatory lesions. However, vascular changes like dilation and congestion of the *Lamina Propria* are characteristic findings in the small intestine. Among these rather unnoticeable microscopic lesions, apoptosis of the jejunal epithelial cells and crypts in the presence of nuclear debris is a

common finding. In some cases, the presence of nuclear debris in the absence of inflammatory cell infiltration may be caused by post-mortal changes or programmed cell death. Occasionally, the intestinal mucosa is characterized by a fusion or destruction and even loss of villi. However, these results need to be interpreted carefully because their occurrence was not constantly seen in all examined cases [6, 16].

4.2 Overview of possible causative agents

There are some well-known infectious causes that are responsible for many digestive disorders in rabbits: the bacterial agents *Escherichia coli* and *Clostridium spp*. As a parasitic infectious cause, we usually find *Eimeria spp*. responsible for digestive disorders [5]. More specifically, the most commonly isolated strains of bacterial pathogens of the digestive tract were enteropathogenic *E. coli* strains (mostly serotype O15: K-: H- and O103: K-:H2), *Clostridium spiroforme, Clostridium piliforme* and *Pasteurella multocida* [16]. Different trials attempted to reproduce ERE with *Clostridium perfringen*s strains and their *alpha-toxins* since they were isolated from the intestinal contents of ERE rabbits, but none of those attempts reproduced the clinical signs of the disease successfully [16]. The genes responsible for alpha, beta2, and theta toxins were identified in *C. perfringens*, but these toxins are typically associated with toxic gastro-intestinal infections and lead to necrotic tissue damage which is not seen in cases of ERE [6].

Attempting to determine the aetiological cause of ERE, a study has fractionated the inoculum TEC4 with two different techniques: firstly, centrifugation on a discontinuous sucrose gradient and secondly cell adhesion. Following the fractionation, two fractions were used to inoculate SPF rabbits and analyzed with standard bacteriological procedures. This resulted in a reproduction of ERE with both fractions. This leads us to believe that viruses can be excluded as the causative agent of the disease since viral particles remained in the supernatant fraction, which did not reproduce any symptoms, and are absent in a different fraction that reproduced the disease [8].

During bacterial examinations of the fractions reproducing ERE, four species that had not been determined in TEC inoculums before were identified: *Mannheimia haemolytica, Brevundimonas vesicularis, Sphingobacterium spiritivorum* and *Gmella morbillorum*. The

first-mentioned is a primary pathogen causing pneumonia, mastitis, and septicaemia in ruminants. The other three are environmental bacteria and are responsible for nosocomial infections. Subsequently, we can exclude them from being responsible for producing ERE until proven otherwise. Furthermore, the fractionation of the TEC4 inoculum resulted in the absence of Gram-negative bacteria in one of the fractions reproducing the disease [19].

Clostridium perfringens plays a crucial role in the pathogenesis of ERE [20]. Other studies have attempted to reproduce ERE with the inoculation of different *Clostridium perfringens* strains into SPF rabbits which also ended up being unsuccessful. This results in an ongoing debate about whether the proliferation of *Cl. Perfringens* in the caecum is a consequence rather than a cause of ERE. However, this study revealed that high numbers of *Clostridium perfringens* are associated with clinical symptoms of ERE and the cause of high mortality [21]. Comparative studies have shown that there are *Cl. Perfringens* strains which induce caecal impaction and high mortality and others which do not. This difference is explained by the production of soluble proteins with mucinase activity by ERE-related *Clostridium perfringens* strains [20].

Knowing that ERE is a multifactorial disease, research teams have detected an over-representation of mucinase-producing bacteria in ERE-affected individuals. Species like *Bacteroides thetaiotaomicron* and *Akkermansia muciniphila* were significantly increased in diseased rabbits [7, 20, 22].
The genus most frequently detected in animals during the age of 25 days was *Bacteroides*. Physiologically, the concentration of this genus decreases frequently throughout the life of the rabbit. However, it is characteristically increased in animals diseased with ERE [20]. Metataxonomic studies showed changes in different bacterial species such as *Clostridium spp., Bacteroides spp., Ruminococcus spp.* [23]*, Akkermansia spp.*[17]*,* and a number of other identifiable and also uncategorized organisms [7, 24]. The results are stated in the following graphics.

BÄUERL ET AL (2014) used the SILVA database to figure out the most common phyla in caecal samples of healthy rabbits, diseased rabbits, and healthy rabbits treated with antibiotics. The following **Figure 3** displays the results. The most commonly occurring phylum in healthy rabbits (marked as “control” in **Figure 3**) were *Firmicutes* with 78.25% of all total reads, *Bacteroidetes* with 15.75% and *Verrucomicrobia* 2.40%, and *Tenericutes* with 2.39% of all reads. The most occurring phylum in all rabbits was *Firmicute*s followed by *Bacteroidetes*. The

class *Clostridia* (phylum *Firmicutes*) was more prominent in healthy groups than in ERE rabbits. The class *Bacteroidia* (phylum *Bacteroidetes*) was most abundant in ERE rabbits among the three groups. Additionally, ERE rabbits presented a significant increase in the *Proteobacteria* phylum, especially in the *gamma-Proteobacteria* class. The number of bacteria belonging to the *Verrucomicrobiae* phylum was also increased in ERE rabbits. This may be due to the increased counts of the genus *Akkermansia* in this group. Furthermore, there was a decrease observed in bacteria belonging to the *Ternericutes* phylum in the ERE group [17].

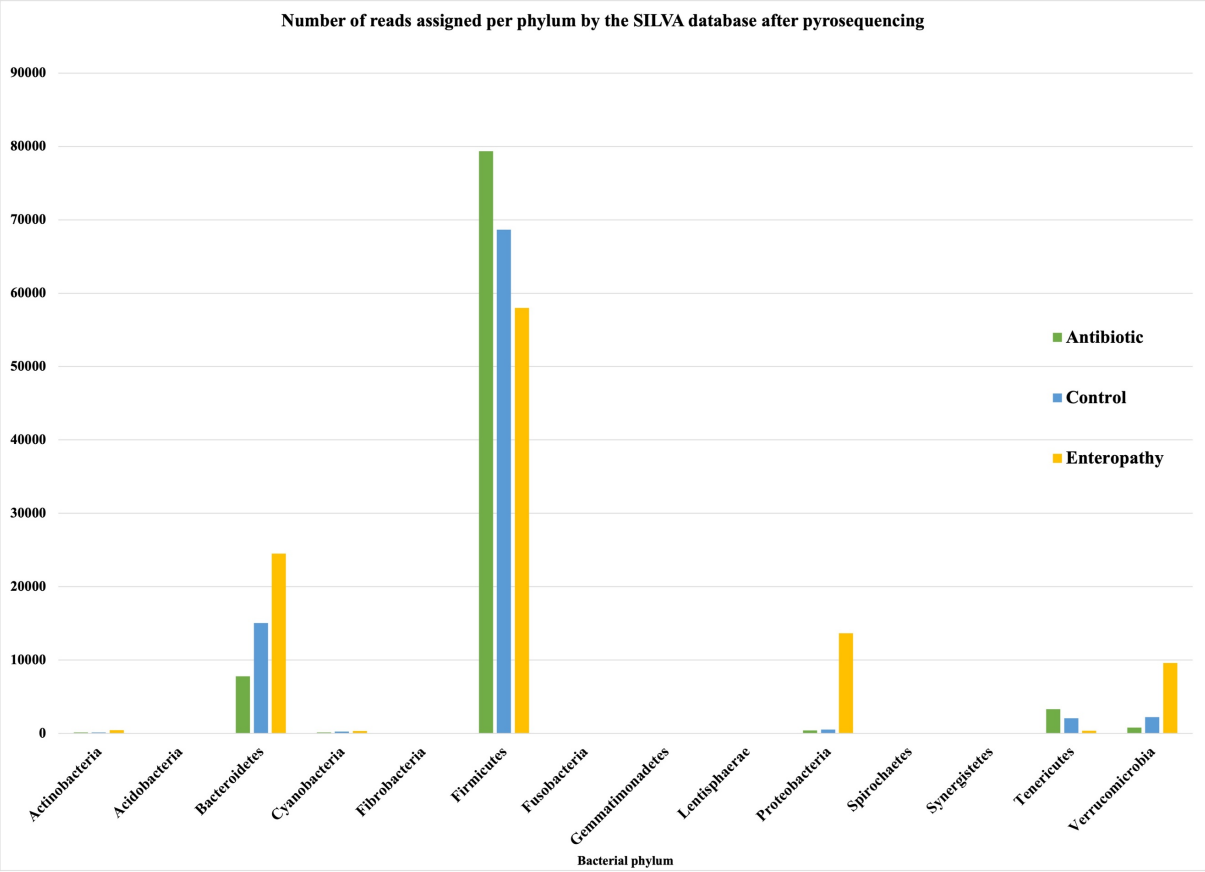

Figure 3: Number of reads assigned per phylum by the SILVA database after pyrosequencing [17].

Own illustration by Chiara Feige.

Among the 90 different genera discovered in caecal samples in BÄUERL ET AL's trials, the most occurring genera of healthy rabbits were *Alistipes* at 5.63%, *Ruminococcus* at 4.02%, *Akkermansia* at 2.40% and *Subdoligranulum* with 2.28% of all total reads. **Figure 4** shows a clear reduction after antibiotic treatment of the *Alistipes,* and *Subdoligranulum* and *Clostridium* genera which belong to the *Bacteroidetes* phylum and the *Firmicutes* phylum respectively. The most frequently occurring genera in the healthy groups and antibiotically treated group were

Ruminococcus and *Alistipes* and *Bacteroides* at 12.45%, *Akkermansia* at 8.40%, *Escherichia* at 8.25%, *Rikenella* at 3.40% and *Clostridium* at 1.24% of all total reads in the ERE group. The genus *Escherichia*, belonging to the *Proteobacteria* phylum, showed an increase in the group of diseased rabbits while it was not portrayed in either of the other groups [17].

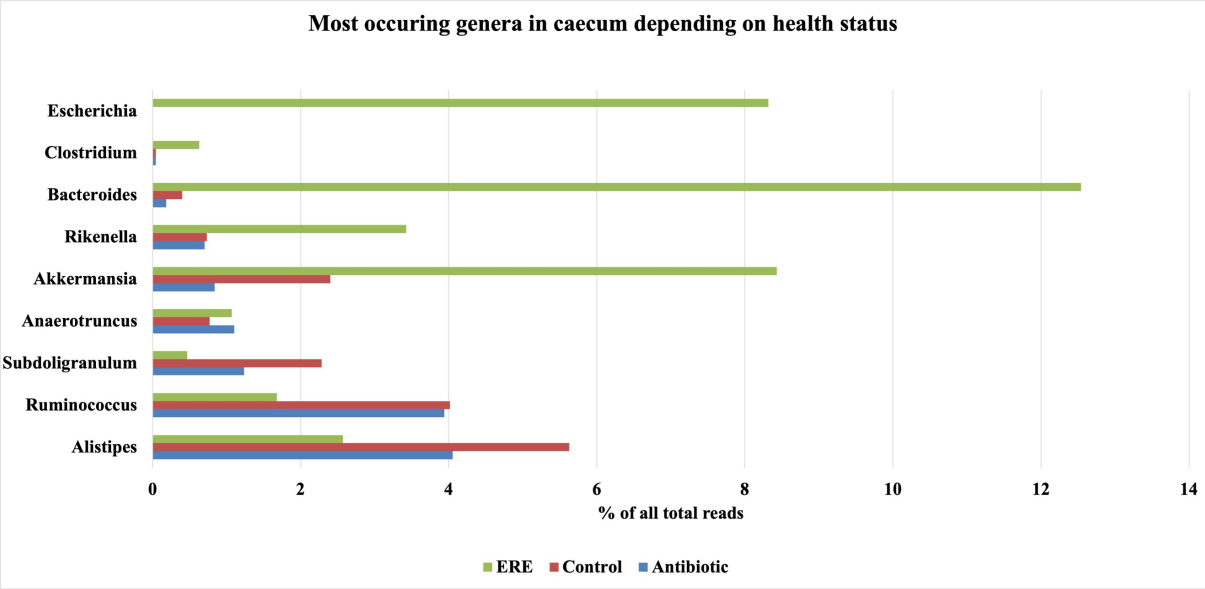

Figure 4: Most occurring genera in rabbit caeca depending on their health status [17]. Own illustration by Chiara Feige.

Taxonomical results from sequencing caecal microbiota of healthy rabbits at the age of 28 days, meaning pre-weaning kits, showed 81% of the identified bacterial agents within the *Firmicutes* phylum and 18% of the *Bacteroidetes* phylum (**Figure 5**). *Tenericutes, Proteobacteria* and *Verrucomicrobia* were only observed in less than 1% of the total sequences. Within the *Firmicutes* phylum, the most dominant families were *Ruminococcaceae* (48%) and *Lachnospiraceae* (16%). The *Bacteroidetes* phylum was dominated by *Bacteroidaceae* (10%) and *Rikenellaceae* (3.5%) families [24].

Healthy post-weaning rabbits at the age of 38 days were characterized by 93% of the identified bacterial agents in the *Firmicutes* phylum and only 5% in the *Bacteroidetes* phylum. The former was represented by an increased number of *Ruminococcaceae* (52%) and *Lachnospiraceae* (23%) (**Figure 6**). The compared ages only varied significantly in the *Bacteroides, Parabacteroides and Clostridium* genera [24].

Diseased rabbits (marked as “not healthy” in **Figure 5** and **Figure 6**) at the age of 28 days displayed a significant decrease in the *Firmicutes* phylum with only 60%. Within the phylum,

the *Ruminococcaceae* family was represented with 24% but showed a decrease in the *Ruminococcus* genus. *Lachnospiraceae* family was however increased. *Bacteroidetes* phylum was represented with an increased amount which enhanced up to 40%, especially within the *Bacteroides* and *Clostridium* genus [24].

Not healthy rabbits at the age of 38 days showed an increase in the *Firmicutes* phylum by 7% whereas the *Bacteroidetes* phylum decreased to 30% (**Figure 5**). However, the number of *Bacteroides* genus increased ten-fold whereas the number of *Ruminococcus* genus decreased (**Figure 6**) [24].

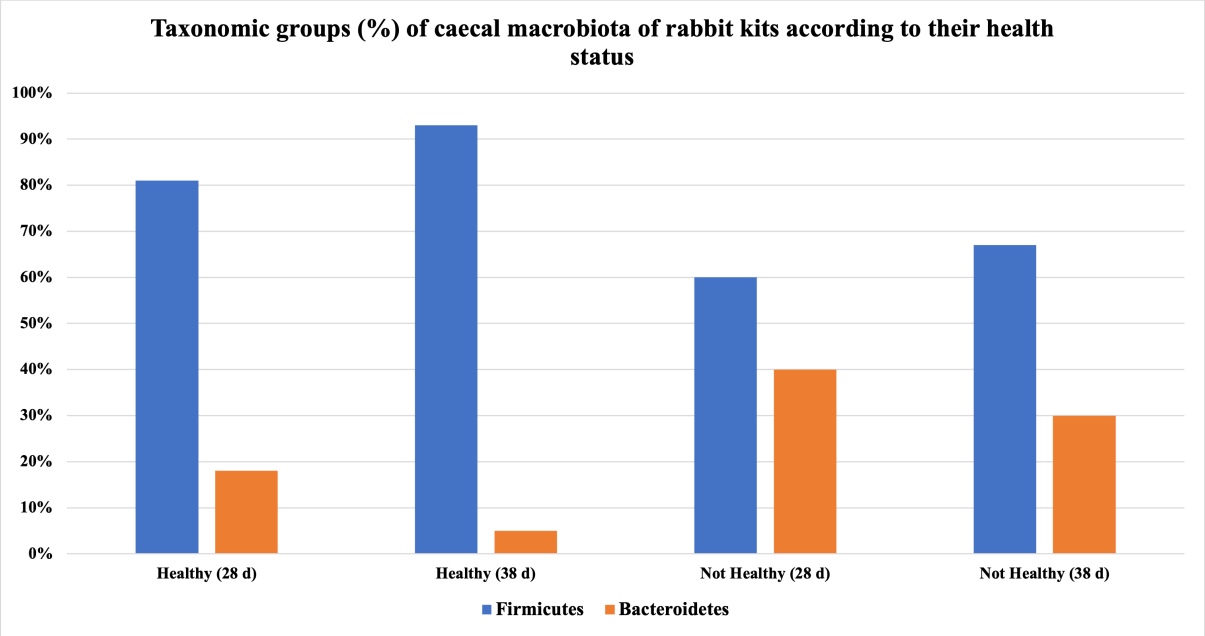

Figure 5: Percentage of taxonomic phyla found in caecal microbiota of rabbit kits according to their age and health status [24]. Own illustration by Chiara Feige.

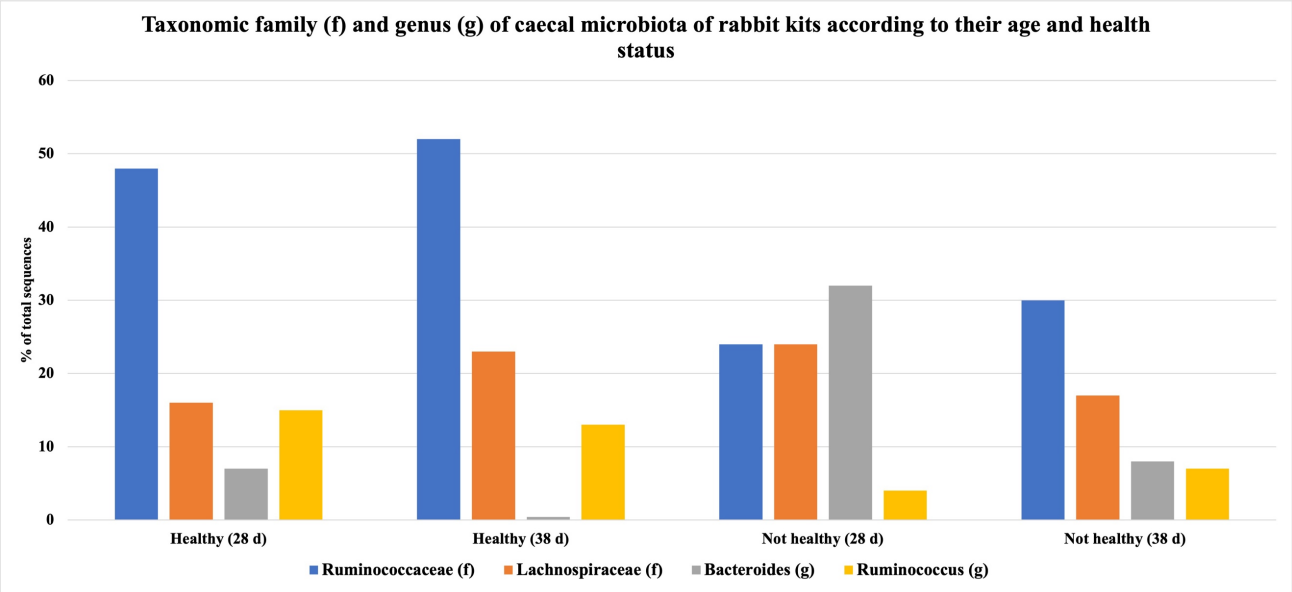

Figure 6: Taxonomic family (f) and genus (g) of caecal microbiota of rabbit kits according to their age and health status [24]. Own illustration by Chiara Feige

A metataxonomic study of 2020 carried out to compare the gastrointestinal microbiota of rabbits diseased with ERE and healthy rabbits revealed many similarities with previously mentioned studies but also uncovered some differences between earlier trials. **Figure 7** and **Figure 8** demonstrate the abundance of phyla, families, and genera appearing in the caecal contents of healthy ("ERE-") compared to the ones appearing in diseased ("ERE+") rabbits. The most occurring phyla in diseased rabbits were *Firmicutes, Bacteroides* and *Verrucomicrobia* while the most occurring phyla in healthy rabbits were *Firmicutes, Verrucomicrobia,* and *Bacteroidetes*. In addition, several unidentified sequences were recorded which were slightly higher in diseased individuals than in healthy individuals (**Figure 7**). Especially ERE-positive rabbits displayed an increased abundance for certain identifiable bacterial genera. *Akkermansia* (phylum *Verrucomicrobiota), Bacteroides* (phylum *Bacteroidetes*), *Cloacibacillus* and *Synergistes* (phylum *Synergistota), Clostridium, Saccharimonas, and Erysipelatoclostridium* were especially increased in diseased rabbits. Additionally, ERE-positive rabbits showed a reduction of the genera *Subdoligranulum* and *Eisenbergiella* and in the families *Ruminococcaceae* and *Lachnospiraceae* (**Figure 8**) [7].

However, in contrast to BÄUERL ET AL'S (2014) results presented in **Figure 3** and **Figure 4**, the metataxonomic study of PUÓN-PELÁEZ ET AL (2020) has not recorded the occurrence of the genus *Escherichia*, nor has it shown any changes in the corresponding phylum *Proteobacteria* [7].

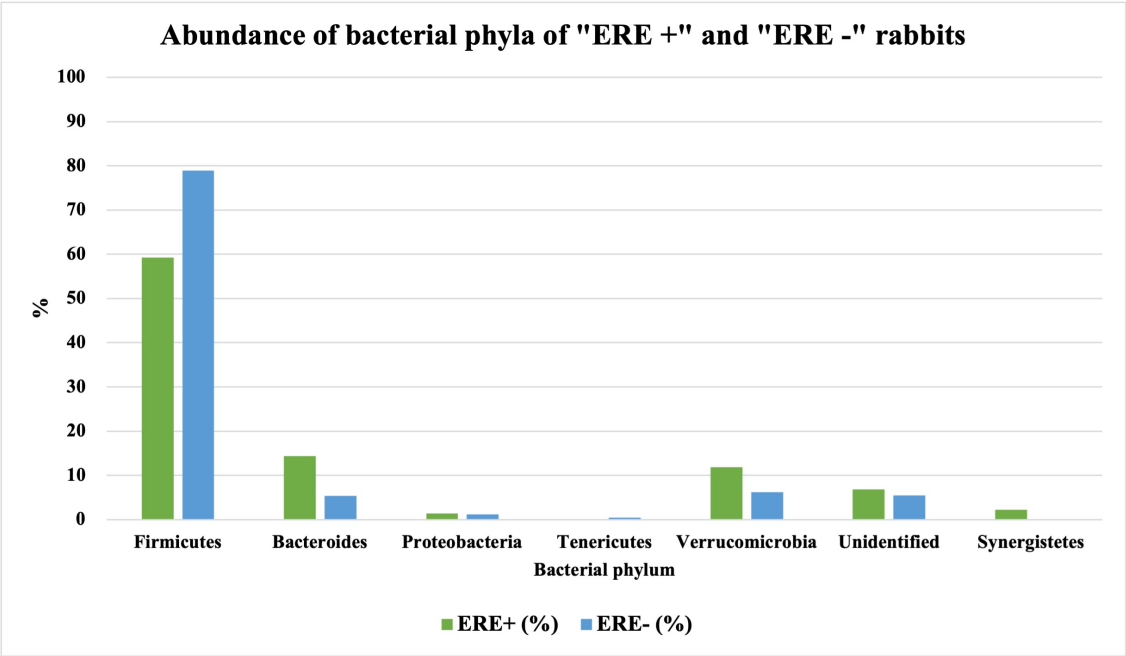

Figure 7: Abundance of bacterial phyla in caecal contents of healthy ("ERE-") and diseased ("ERE +") rabbits [7]. Own illustration by Chiara Feige.

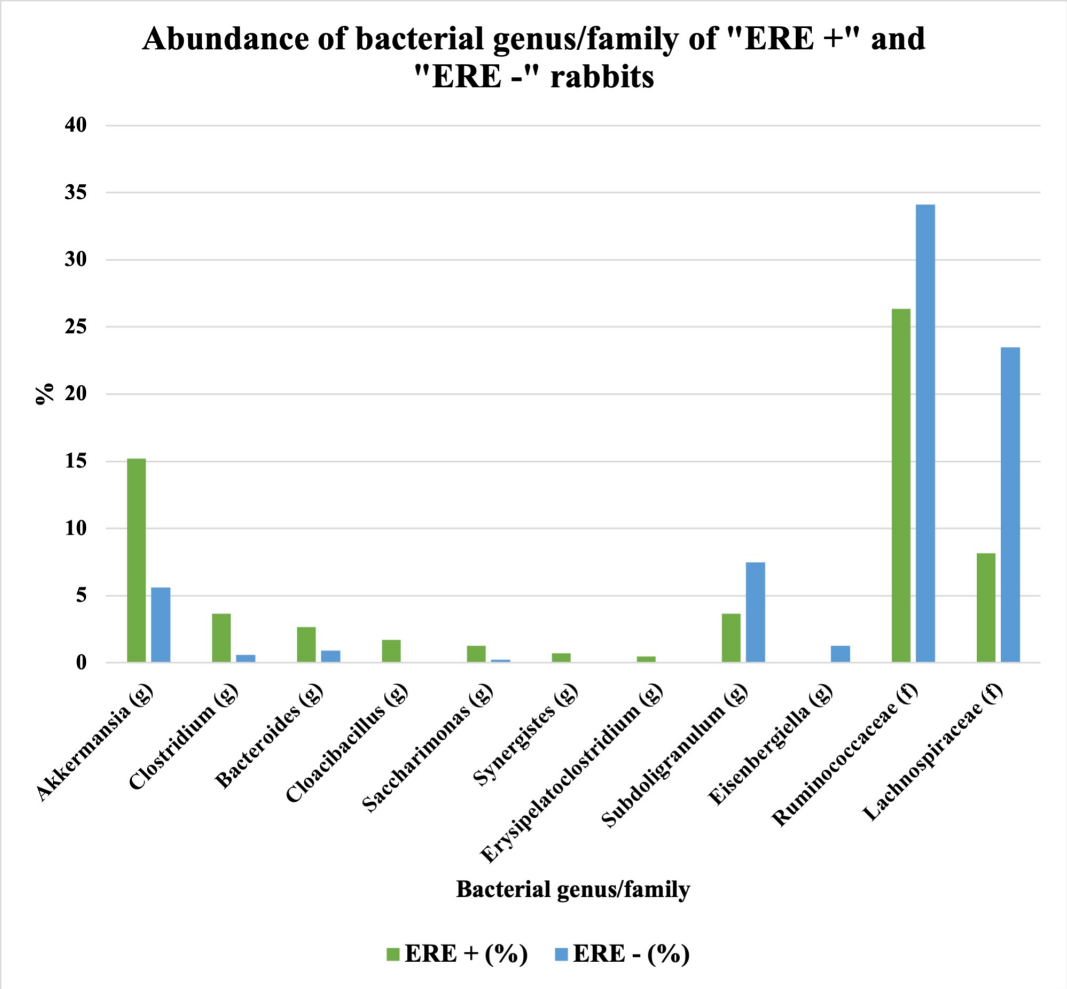

Figure 8: Abundance of bacterial genera and families occurring in caecal samples of healthy ("ERE –") and diseased ("ERE+") rabbits. (g): genus, (f): family) [7]. Own illustration by Chiara Feige

Despite the research carried out to this date, the bacterial species causing ERE is still unknown. Either this species is not cultivable on our common culture media, or its in vitro growth is inhibited by certain other factors like other bacterial species occurring in the TEC inoculum [19].

5 Recent microbiological advances

5.1 Latest research findings on the microbiological causes of ERE and advancements in diagnostic techniques

Various methods, both individually and in combination, were utilized to pinpoint and isolate the pathogen of ERE. These methods dealt with techniques such as concentrating and purifying it using density gradients, studying it under electron microscopes, growing it in cell cultures, and utilizing molecular biology techniques [6].

The firstly used cell cultures to culture the pathogen in different cell lines only gave unsatisfactory results due to the shortage of information about suitable cell lines and the lack of general knowledge about the causative biology behind the disease. Cell cultures as diagnostic tools were later abandoned and replaced by purification methods with the help of ultracentrifugation [6].

5.2 PCR

Polymerase chain reaction (PCR) is a frequently used tool in research. However, in contrast to many previous investigations, PUÓN-PELÁEZ ET AL (2020) have not undergone one round of PCR before sequencing, instead, the sequencing happened directly from the genomic DNA. The so-called "universal" primers, which are originally believed to work for all intended targets, may not always perform as universally as initially thought, which may lead to potential difficulties in amplifying all the desired targets. Additionally, since PCR is not an error-free tool, it has to be regarded with caution since amplification errors may lead to fundamental sequencing mistakes [7, 25, 26].

5.3 RAPD

Studies have compared virulent and non-virulent inocula with the help of Random Amplification of Polymorphic DNA (RAPD). The objective was to determine specific parts of the virulent fractions that could be helpful in recognizing bacterial agents causing ERE. The identified agents were isolated and used to inoculate SPF rabbits. The occurrence or prevalence of the particular genetic sequences in rabbits that passed away due to ERE or other gastrointestinal issues was calculated. One specific gene (R6B sequence that lies within the yijP gene) determined was found in *Enterobacteriaceae*, but none of these species have been

isolated from TEC4 and TEC4 inocula. For further studies, TEC4 was plated on a culture medium in aerobic and anaerobic conditions and incubated, the grown cultures were scraped and specific PCRs were performed to identify the previously isolated genetic sequence R6B. Two bacterial isolates turned out to be positive for R6B and were identified as *Staphylococcus epidermidis*. One *S. epidermidis* strain was used to inoculate 18 six-week-old SPF rabbits. Another group of SPF rabbits was inoculated with the TEC4 inoculum as the positive control group. As a result of the inoculation with *S. epidermidis*, no ERE-typical clinical signs like a decrease in daily weight gain or diarrhoea were observed. Only the positive control group (TEC4) was able to reproduce these typical lesions. In conclusion, the genetic sequence identified with the help of RAPD profiles found in *S. epidermidis* was unable to reproduce ERE. TEC3 and TEC4 and their fractions still remain solely able to reproduce ERE [27].

5.4 DGGE

Denaturing Gradient Gel Electrophoresis (DGGE) is a method employed to separate DNA fragments of moderate length by their distinctive melting properties. This technique is often utilized for detecting individual genetic variations at the single-nucleotide level, eliminating the necessity for DNA sequencing. Additionally, it serves as a molecular fingerprinting approach for intricate ecosystem communities, especially when combined with the amplification of microbial 16S rRNA genes [28]. The 16S rRNA molecule serves as the critical structural element within the 30S ribosomal subunit of bacteria, playing an essential role in starting the process of protein synthesis [29]. Results of DGGE performance in a study confirmed differences between virulent and non-virulent fractions of fractionated inocula which supports the theory of ERE having a bacterial aetiological background, but this needs to be investigated further to gain knowledge about DNA sequence and to explain the difference [8].

5.5 Role of bacterial flora and gut health in ERE

The normal bacterial flora of healthy rabbits is characterized by mostly strictly anaerobic bacterial species such as the phyla *Firmicutes* (mostly *Ruminococcae* & *Lachnospiracae*), *Bacteroidetes* (*Rikenellaceae* mostly), *Verrucomicrobiota* and *Tenericutes*. A study has determined the change of bacterial genera during an ERE infection and during antimicrobial treatment. The results are documented in **Figure 3** and **Figure 4** and they showed a dominance of the genera *Ruminococcus, Alistipes* and *Akkermansia* in healthy untreated rabbits and a slight increase in the *Ruminococcus* genus and a decrease in the *Akkermansia* genus during

antimicrobial treatment. The genera that were mostly occurring during ERE infections were *Clostridium, Bacteroides, Alistipes and Akkermansia* [17].

The fact that there were no significant differences in the microbiota of the untreated control group and the antibiotically treated group might be caused by the extended usage of preventive antimicrobials in the experimental farm. This scenario might lead to the establishment of a homogenous environmental population within the farm, which could eventually inhabit and spread among the rabbit population. However, this homogenous bacterial environment may help with the detection of bacterial organisms involved in the pathogenesis of ERE. The key characteristic of ERE's cecal microbiota is a significant imbalance and a decrease in the taxonomic variety [17].

Important components of the healthy microbiome include *Ruminococcus* which is decreased in ERE, even though it is a mucin-degrading genus, and *Alistipes*, but both are responsible for the degradation of vegetables and production of short-chain fatty acids. These two genera could be used to make probiotics since they seem to be essential to the caecal microbiome [17].

6 Management and control

6.1 Impact of hygiene measures

Studies have shown that after the weaning stage, hybrid populations in commercial rabbit farms have portrayed a mortality rate exceeding four times that observed in identical breeds subjected to controlled conditions in randomly selected sample testings [30–33]. This substantial imbalance strongly implies that environmental factors, such as variations in the way rabbits are raised and managed, can influence both health and mortality rates. To some extent, the mortality seen in the domestic rabbit farming industry can be caused by environmental factors, including management practices that fail to align with the behavioral and nutritional needs of this species [33]. Preventing preventable deaths is a fundamental aim of animal welfare regulations [33, 34]. Therefore, the goal is to identify causal connections between certain aspects of current farming practices and mortality and health issues in domestic rabbits [33].

The constant exposure of domestic rabbit kits to 16 hours of daylight or artificial light increases their activity compared to those exposed to only 8 hours of light [33, 35]. This, combined with shorter feeding times when they consume pelleted feed, rather than green forage or hay, may contribute to abnormal behaviors in kits, such as fur chewing (trichophagy) [33, 36].

The risk of infection is a significant concern when rearing rabbits in deep litter. Studies have shown that a high percentage (92-94%) of does excrete coccidial oocysts when kept in such conditions [33, 37, 38]. Furthermore, coccidiosis-infected kits continue to excrete oocysts when reared on deep litter, whereas this stops after about one month when they are raised on wire mesh [33, 39]. Additional research indicates that kit mortality due to enteropathies increases significantly (34% to 100%) when animals are reared on deep litter compared to housing in cages with wire mesh floors [33, 40]. Additionally, the microbiome of the mother doe defines the microbial profile of her litter, meaning, a disturbed microbial flora will affect her whole litter and therefore predispose it to enteropathogenic diseases [7, 41].

6.2 Impact of fibre and protein content of the diet

Since ERE is displayed as a gastro-intestinal disorder, suitable nutrition during the post-weaning time might play a big role in the prevention of the disease. Insoluble fibre (NDF) is known to decrease the caecal retention time and therefore also the microbial growth in the hindgut. However, a surplus of NDF has a harmful effect on the intactness of the jejunal mucosa at 45 days of age. Subsequently, reduced fibre content of the diet may act as a predisposing factor for ERE infections [17, 21].

In a trial, recently weaned rabbits were fed a high-fibre (HF) diet and a low-fibre (LF) diet. The diets were developed to have similar amounts of protein (150g/kg), soluble fibre (120g/kg), and insoluble fibre (130g/kg). This special diet was given to 40 different litters from day 21 of lactation until 56 days of age which is when the experimental period ended. Results of the study in terms of post-weaning mortality showed that an increased amount of NDF resulted in higher mortality of fattening rabbits in poor hygienic conditions and a structural alteration of the intestinal mucosa [21].

Wild rabbits, being leaf-eating folivores, choose the most digestible and protein-rich parts of green vegetation [33, 42] while avoiding larger, older shoots and stems with low-digestible fiber (lignocellulose). This selective feeding results in their consumed food having higher protein and nutrient content compared to the average vegetation. For example, the protein content of their preferred forage is about three times higher than the protein content of the plants typically found in their environment. Other less preferred food sources like bark, twigs, and grass roots are only consumed during periods of food shortage [33, 43].

Domestic rabbits also favor plant material that contains a high amount of leaves and has a higher protein content [33, 44]. Usually, fresh green food or roughage alone isn't enough to provide all the nutrients needed for the growth of kits from domestic breeds. This is because, unlike wild rabbits, domestic breeds tend to grow much bigger as adults. The reason for this is that as domestic rabbits get larger, their digestive systems become relatively less efficient, which means they can't extract as many nutrients from their food as efficiently as smaller breeds [33, 45, 46].

6.3 Restriction diets as preventive measures

Feed restriction is a commonly used method to prevent or lower the chances of digestive pathologies in rabbits in their growing phase [47]. It is generally known to improve nutrient digestion as a consequence of the increased residence time of the feed in the gastrointestinal tract [47, 48]. Especially around weaning time, young rabbits are very prone to gastro-enteric pathologies which can cause high mortality. Usually, there are different ways to restrict the feed intake: in terms of time intervals [47, 49] (usually 1-3 weeks after weaning) or in terms of amount (restricting a certain % of feed in connection to a standard ad libitum intake) [47, 50, 51].

During a trial, 256 Hyla-rabbits were divided into two groups which were given the same commercial concentrate but fed differently: one group was fed ad libitum (group AL) while the restricted group (RES) received 90% of the ad libitum intake from weaning (35 days of age) until the day of slaughter (85 days of age). Mortality, group feed intake, and live weight were documented on a daily basis [47].

Throughout the trial, feed intake of the RES group was an average of 11.1 % less than the AL group. Live weights were significantly higher for the restricted group on day 56 and day 63, while rabbits of both groups showed no significant difference in their slaughter weight.

Taking into account the whole trial, daily weight gain (DWG) was not considerably different between the two groups. According to other trials, the food conversion rate (FCR) was statically lower in the RES group, while it was significantly higher in this trial [47].

In conclusion, a feed reduction of 10% throughout the entire production cycle of rabbits improved the digestive efficiency of the nutrients and did not alter the health status or growth of the animals [47].

A different trial, also carried out to investigate the impact of feed restriction in the growing phase of rabbits on digestive health and growth, confirms the positive effect of a restrictive diet after weaning. During the post-weaning period of growing rabbits, the observed mortality was notably lower from a restricting value of 80% of the AL feed intake. It has proven to be most beneficial to reduce the AL intake by up to 70% to have a maximum decrease in morbidity. However, these results did not continue when the feed intake was changed back to AL feeding (day 54 of age until slaughter). Additionally, this trial detected that the most beneficial results were achieved when they applied a feeding program with 20 days of restricting the AL feed

intake by 20% and then 18 days of unrestricted AL feed intake. All in all, this feeding system can be regarded as beneficial in terms of feeding costs and decreased losses of young rabbits [9].

6.4 Usage of preventive antimicrobials

Since ERE appeared in Europe, antibiotics have been a commonly used form to treat and prevent the disease [1]. Bacitracin, an antibiotic agent from the polypeptide group, has proven to be an effective food additive in terms of preventive and curative treatment of ERE [52].

The following trial investigates the efficacy of bacitracin solved in drinking water in comparison to the efficacy of bacitracin used as a feed additive. Furthermore, it was designed to compare the curative and the preventive usage of bacitracin solved in the drinking water [53]. 168 weanlings of 32 days of age were divided into 4 groups (A,B,C,D) of 42 rabbits each. They were kept in cages of each 7 individuals in one common room with ad libitum access to feed. Inoculation happened on day 42 of age via the TEC3 inoculum. All groups were fed concentrates without any antibiotic additives except for group B which received bacitracin 100ppm mixed in the feed from day 32-60 of age. Group C received a preventive treatment of bacitracin via drinking water in a dosage of 0.675 g/l (2835 IU/L) 9 days before inoculation and 10 days after inoculation. Group D was treated curatively also with bacitracin solved in the drinking water for 14 days after the first symptoms of the disease appeared at 45 days of age. Throughout the treatment of Groups D and C, the medical solution was renewed every day. Group A remained the untreated control group [53].

Body weights and feed consumption were controlled regularly as well as mortality and water consumption with estimated consumption of the solved bacitracin [53].

Results confirmed the efficiency of bacitracin used as a treatment for ERE [12, 52, 53]. Using bacitracin in the drinking water as preventative treatment was as efficient as 100ppm bacitracin given as feed additive during the acute period of the disease. The curative use of bacitracin in drinking water for 14 days reduces morbidity and mortality after symptoms appeared in comparison to the untreated control group, but it was not as efficient as preventive usage. This appeared to be caused by the extremely fast onset of clinical signs after contamination (less than 48 hours) [53].

Bacitracin has proven to be an effective food additive at a dose of 100 mg/kg in terms of preventive and curative treatment of ERE [52]. However, due to a European ban (EU reg. 2821/98), Zinc-bacitracin was banned as a feed additive. The following study has tested the efficacy of bacitracin in the drinking water of chronically infected rabbits.

The study divided 384 rabbits into three different groups of freshly weaned, 30-day-old rabbits. They were kept in cages of four rabbits each, which were sorted into six rows of 16 cages in one room. Each row received its own water reservoir. Each group consisted of 128 rabbits; one group was untreated (negative control group) while the two other groups were treated with bacitracin through drinking water at a dose of 420 IU bacitracin per kg body weight (BW) per day. The first group was treated for 14 days (T14) whereas the second group was treated for 21 days (T21). The treatment was scheduled to start as soon as at least one animal with clinical symptoms of ERE had died. The given dosage of medication was adjusted to the measured weight of the animals, the number of rabbits alive with a daily weight gain of 40g/day/rabbit, and it was then further diluted into a volume matching 70% of the expected daily water intake [12].

During the study, the mortality in the untreated control group was 26.6% while it was 13.5% in T14 and 12.6% in T21. There were additional distinctions made considering mortality with signs of ERE and mortality without signs of ERE during necropsy. Keeping these values in mind, the absolute ERE-caused mortality was 14.1% in the untreated group, 4% in T14 and 6.3% in T21 [12]. Mortality in the untreated control group was rather occurring during the earlier stages of the trial while mortality in the medicated groups was increased in the later stages of the trial, when the treatment ended. All in all, both medicated groups performed significantly better than the untreated control group. Moreover, T21 showed notably better results than T14 as T14 had almost twice the number of affected animals than T21 [12].

In conclusion, both medicated groups performed notably better in terms of mortality, clinical symptoms, and performance data than the untreated control group. and were able to control the infection immediately after the start of the treatment. However, some days after stopping the treatment period, clinical symptoms of the disease and mortality were observed in the medicated groups. This was considered to be caused by a recontamination of the untreated control group kept in the same room as the treated groups [12].

To conclude, a 21-day treatment could be considered a treatment option for ERE due to its exceptional reduction of clinical symptoms compared to a 14-day treatment with soluble bacitracin in the drinking water [12].

However, it is important to mention that antimicrobial drugs not only have a beneficial effect on the individual's gut health, but that it can also have harmful consequences. Antibiotics can alter the fermentative properties of the hindgut and can so induce antibiotic-associated diarrhoea [54]. In addition to the already disturbed microbial balance, it can induce enterotoxaemia. This study investigates the efficacy and tolerability of an early administration of valnemulin given as a feed additive during ERE infection [55]. Valnemulin belongs to the antibiotic group of pleuromutilins and acts as a protein synthesis inhibitor by binding to the 50S ribosomal subunit. In this study, valnemulin was given at a dosage of 20 and 35 ppm as a feed additive and its effects on mortality, growth, and FCR were observed. Additionally, it was investigated whether any adverse effects occurred because of the antibiotic usage [55].

1149 recently weaned rabbis of the ages of 35 ± 2 days were kept in groups of four individuals per cage. All animals in the same cage received the same treatment. They were randomly sorted into three different treatment groups as there were: 2 medicated groups which were given either 20 ppm or 25 ppm of valnemulin from day 1 to 21 and one untreated control group. Treatment day 0 was defined by the first rabbit which was diagnosed with typical ERE clinical signs. None of the groups received any medicated feed from days 21-28. Throughout the medication period, rabbits received valnemulin mixed in the feed and their daily feed consumption was measured. The results of this study showed a significant difference in mortality between the medicated and untreated groups. The mortality due to ERE in the untreated control group was 23% while it was 11% and 7.6% in the groups that received 20 ppm and 35 ppm valnemulin respectively. Throughout the entire study, the different groups showed no significant differences in terms of average daily weight gain and FCRs, only temporary fluctuations between the groups were observed. In addition, there were no adverse effects like antibiotic-responsive diarrhoea examined throughout the medication period. Due to its advantageous effects such as a significant reduction in mortality in the absence of an alteration of the FCR, daily weight gain and adverse effects, an oral administration of valnemulin as a metaphylactic treatment of 20ppm and 35 ppm is an effective and secure way to treat and control ERE in recently weaned rabbits [55].

6.5 Usage of probiotics as a preventive measure

To avoid certain unwanted side effects of the usage of antimicrobials in the nutrition of rabbits such as antimicrobial resistance or antibiotically caused diarrhoea, the administration of probiotics has increased. Probiotics consist of live microorganisms which affect the gut's microflora positively [56–59]. The increased usage of antimicrobials as growth promotors has led to the development of more and more multi-drug resistant bacterial strains, which can pose a potential threat to the human microbiota [56, 60, 61]. Therefore, the European Union has banned antimicrobials as growth promotors since 2006 [56].

In this study, rabbits were fed different autochthonous strains of *Enterococcus spp*. and *Escherichia coli* throughout a 25-day trial to examine the effect on faecal microbiota and antimicrobial resistance profiles. The control group was fed a diet consisting of commercially fed concentrates and antibiotics such as colistin, oxytetracycline, and valnemulin. The concept of probiotics is based on the administration of live and beneficial microbial organisms into the intestinal microbiome. Introduced microorganisms are supposed to outcompete opportunistic bacterial strains in terms of colonization space and they are expected to reduce the susceptibility to enteric diseases [56, 62, 63].

Results of the 25 day-trial showed that growth rates of animals that were fed with antibiotically medicated feed and animals that received probiotics were not significantly different. Furthermore, multi-drug resistant strains of *Enterococci* and *E. coli* were more frequently observed in faecal material of rabbits treated with antimicrobials. The usage of *E. coli* as a probiotic must happen with care due to possible adverse reactions caused by enteropathogenic (EPEC) *E. coli* strains. In conclusion, *Enterococci* strains might be a more effective probiotic than *E. coli*, but they lack the ability of long-term colonization since they disappeared from faecal material as soon as the administration stopped or the housing changed [56].

7 Future perspectives

Potential research areas in the context of ERE could investigate whether a causative agent(s) is found if searched in earlier stages of the life of rabbits. Considering that ERE can be replicated by both fiber-deficient diets and cecum ligation, it's reasonable to deduce that there may be an infective agent, physiological anomaly, or even an unexpected occurrence in some young rabbits that disrupts their intestinal motility and the ileo-caecal valve. This disruption inhibits the normal emptying of the cecum, resulting in the problematic overgrowth of bacteria in the caecum. Therefore, it can be suggested that to truly understand the root cause of ERE, research should focus on investigating earlier stages, even in rabbits that currently appear healthy [17].
Furthermore, it might be valuable to isolate bacterial species with sequences that have not been identified yet to assess their abilities to cause infection. For example, we could explore different strains from the *Bacteroides* genus or two strains within the *Lachnospiraceae* family, which collectively made up 13.8% of all the identified sequences in ERE-affected rabbits [17].
To uncover the reasons behind this illness, particularly since it mainly affects rabbits after weaning, forthcoming investigations should contain a comprehensive study over time. This study should focus on the microbiota of caecotrophs and the immune parameters in young rabbits during this period. Additionally, researchers should explore potential protective factors from does, as well as substances that encourage epithelial and immune development, which young rabbits lack after weaning [17].

8 Conclusion

In conclusion, the study of Epizootic Rabbit Enteropathy has shed light on the multifaceted challenges that the rabbit industry faces. ERE has proven to be a significant health concern, affecting the productivity and well-being of rabbits worldwide. Through investigations, we have achieved a deeper understanding of the complex factors contributing to ERE, including environmental conditions, dietary management, and potential pathogens.

Research has highlighted the critical role of nutrition and husbandry practices in preventing and managing ERE. High-fiber diets, attention to hygiene, and careful consideration of rearing practices are essential in reducing the incidence and impact of this disease. Additionally, the findings emphasize the importance of ongoing collaboration between researchers, veterinarians, and the rabbit industry to develop effective strategies for ERE prevention and management.

Looking forward, the future of the rabbit industry lies in the continued pursuit of improved practices, breeding, and healthcare. We anticipate the development of targeted interventions and guidelines aimed at reducing the prevalence of ERE. Furthermore, the industry would benefit from advancements in rabbit genetics, promoting the breeding of healthier and more resilient animals.

As we move further, the rabbit industry must adapt to changing demands, including a growing emphasis on sustainable and ethical farming practices. There is an opportunity for innovation in the production and marketing of rabbit meat and products, ensuring a more prosperous and secure future for the industry.

To conclude, while ERE displays challenges to the rabbit industry, it also presents opportunities for growth and development. By addressing these challenges and building on the insights gained from the research, the industry can look forward to a future that is both economically viable and socially responsible. Through a commitment to best practices, improved rabbit health, and sustainable farming, the rabbit industry can thrive and contribute to meeting the world's growing demand for high-quality protein sources.

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References

1. Puón-Peláez X-H, McEwan N, Am O-R (2018) Epizootic Rabbit Enteropathy (ERE): A Review of Current Knowledge. Eur Sci J ESJ 14:137–137. https://doi.org/10.19044/esj.2018.v14n36p137

2. Coudert P, Lebas F, Licois D (1997) Une nouvelle pathologie ravage les élevages:-la population se mobilise, la profession se mobilise. Cuniculture 1–8

3. Vandekerchove D, Charlier G, Roels S (2000) A naturally occurring case of mucoid enteropathy in a specific pathogen free (SPF) rabbits. pp 363–368

4. Licois D, Wyers M, Coudert P (2005) Epizootic Rabbit Enteropathy: experimental transmission and clinical characterization. Vet Res 36:601–613. https://doi.org/10.1051/vetres:2005021

5. Bouquin SL, Jobert JL, Larour G, Balaine L, Eono F, Boucher S, Huneau A, Michel V (2009) Risk factors for an acute expression of Epizootic Rabbit Enteropathy syndrome in rabbits after weaning in French kindling-to-finish farms. Livest Sci 125:283–290. https://doi.org/10.1016/j.livsci.2009.05.010

6. Licois D, Coudert P, Ceré N, Vautherot J-F (2000) Epizootic enterocolitis of the rabbit: review of current research. World Rabbit Sci 8:187–194

7. Puón-Peláez X-HD, McEwan NR, Gómez-Soto JG, Álvarez-Martínez RC, Olvera-Ramírez AM (2020) Metataxonomic and Histopathological Study of Rabbit Epizootic Enteropathy in Mexico. Animals 10:936. https://doi.org/10.3390/ani10060936

8. Huybens N, Houeix J, Szalo IM, Licois D, Mainil J, Marlier D (2008) IS EPIZOOTIC RABBIT ENTEROPATHY (ERE) A BACTERIAL DISEASE? In: 9th World Rabbit Congress, Verona, Italy. pp 971–975

9. Gidenne T, Combes S, Feugier A, Jehl N, Arveux P, Boisot P, Briens C, Corrent E, Fortune H, Montessuy S, Verdelhan S (2009) Feed restriction strategy in the growing rabbit. 2. Impact on digestive health, growth and carcass characteristics. Animal 3:509–515. https://doi.org/10.1017/S1751731108003790

10. Marlier D, Dewrée R, Lassence C, Licois D, Mainil J, Coudert P, Meulemans L, Ducatelle R, Vindevogel H (2006) Infectious agents associated with epizootic rabbit enteropathy: Isolation and attempts to reproduce the syndrome. Vet J 172:493–500. https://doi.org/10.1016/j.tvjl.2005.07.011

11. Szalo IM, Lassence C, Licois D, Coudert P, Poulipoulis A, Vindevogel H, Marlier D (2007) Fractionation of the reference inoculum of epizootic rabbit enteropathy in discontinuous sucrose gradient identifies aetiological agents in high density fractions. Vet J Lond Engl 1997 173:652–657. https://doi.org/10.1016/j.tvjl.2005.12.013

12. Maertens L, Cornez B, Vereecken M, Oye SV (2005) Efficacy study of soluble bacitracin (Bacivet S®) in a chronically infected epizootic rabbit enteropathy environment. World Rabbit Sci 13:165–178. https://doi.org/10.4995/wrs.2005.520

13. Duperray J, Guyonvarch A, Laurent J, Adelis R, Haberkorn F, Licois D (2011) Experimental reproduction of Epizootic Rabbit Enteropathy (ERE) with a new inoculum: TEC 2.1

14. Licois D (2004) Domestic rabbit enteropathies. In: Proc. 8th World Rabbit Congress, Puebla, Mexico. pp 385–403

15. Guitian J, Corrales J, Prieto C, Vega M, Cachaldora P, Feranadez P, Hermida M, Sanjuan M, Yus E (2000) An assay of experimental mucoid enteropathy with commercial dry rabbits pellets. In: Proc. 7th World Rabbit Congress, Valencia—Spain. pp 4–7

16. Dewrée R, Meulemans L, Lassence C, Desmecht D, Ducatelle R, Mast J, Licois D, Vindevogel H, Marlier D (2007) Experimentally induced epizootic rabbit enteropathy: clinical, histopathologicaI, ultrastructural, bacteriological and haematological findings. World Rabbit Sci 15:91–102. https://doi.org/10.4995/wrs.2007.602

17. Bäuerl C, Collado MC, Zúñiga M, Blas E, Pérez Martínez G (2014) Changes in cecal microbiota and mucosal gene expression revealed new aspects of epizootic rabbit enteropathy. PloS One 9:e105707. https://doi.org/10.1371/journal.pone.0105707

18. de Rozas Ruiz AMP, Carabaño R, García J, Rosell J, Cano JVD, García JB, Amorós JJP, Saíz JIB (2005) Etiopatogenia de la enteropatía epizoótica del conejo. pp 167–174

19. Huybens N, Houeix J, Licois D, Mainil J, Marlier D (2009) Inoculation and bacterial analyses of fractions obtained from the reference inoculum TEC4 which experimentally reproduces epizootic rabbit enteropathy. World Rabbit Sci 17:. https://doi.org/10.4995/wrs.2009.643

20. Badiola I. , Perez de Rozas A. , Gonzalez J., Aloy N. , García J. , Carabaño R., 2016.- Recent advances in ERE in growing rabbits (Invited paper). Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 491-502.

21. Romero C, Nicodemus N, García-Rebollar P, García-Ruiz AI, Ibáñez MA, De Blas JC (2009) Dietary level of fibre and age at weaning affect the proliferation of Clostridium perfringens in the caecum, the incidence of Epizootic Rabbit Enteropathy and the performance of fattening rabbits. Anim Feed Sci Technol 153:131–140. https://doi.org/10.1016/j.anifeedsci.2009.05.005

22. Macchione I, Lopetuso LR, Ianiro G, Napoli M, Gibiino G, Rizzatti G, Petito V, Gasbarrini A, Scaldaferri F (2019) Akkermansia muciniphila: key player in metabolic and gastrointestinal disorders. Eur Rev Med Pharmacol Sci 23:

23. Huybens N, Houeix J, Licois D, Mainil J, Marlier D (2013) Pyrosequencing of epizootic rabbit enteropathy inocula and rabbit caecal samples. Vet J 196:109–110. https://doi.org/10.1016/j.tvjl.2012.08.014

24. Abecia L, Rodríguez-Romero N, Martínez-Fernández G, Martínez-Vallespín B, Fondevila M (2017) Pyrosequencing study of caecal bacterial community of rabbit does and kits from a farm affected by epizootic rabbit enteropathy. World Rabbit Sci 25:261–272

25. Zhou X, Li Y, Liu S, Yang Q, Su X, Zhou L, Tang M, Fu R, Li J, Huang Q (2013) Ultra-deep sequencing enables high-fidelity recovery of biodiversity for bulk arthropod samples without PCR amplification. GigaScience 2:2047–217X

26. Zaura E (2012) Next-generation sequencing approaches to understanding the oral microbiome. Adv Dent Res 24:81–85

27. Huybens N, Houeix J, Licois D, Mainil J, Marlier D (2011) Epizootic rabbit enteropathy: Comparison of PCR-based RAPD fingerprints from virulent and non-virulent samples. Vet J 190:416–417. https://doi.org/10.1016/j.tvjl.2010.10.010

28. Strathdee F, Free A (2013) Denaturing gradient gel electrophoresis (DGGE). Methods Mol Biol Clifton NJ 1054:145–157. https://doi.org/10.1007/978-1-62703-565-1_9

29. Jay ZJ, Inskeep WP (2015) The distribution, diversity, and importance of 16S rRNA gene introns in the order Thermoproteales. Biol Direct 10:35. https://doi.org/10.1186/s13062-015-0065-6

30. Lange K, Schlolaut W (1981) Vergleichende Untersuchungen der Reproduktions- und Mastleistung von zwei Kaninchenpopulationen. In: Arbeitstagung Dt. Vt. med. Ges. Celle, Germany, pp 54–62

31. Lange K (1985) Untersuchungen über den Einfluss der Rein- und Kreuzungszucht auf die Reproduktions-, Mast- und Schlachtleistung des Kaninchens.

32. Lange K (1997) Herkunfts- Vergleichsprüfung von vier Kaninchenpopulationen auf Reproduktions-, Mast- und Schlachtleistung. Prüfungsbericht, Hess. Landesanstalt f. Tierzucht, Neu-Ulrichstein, Germany.

33. Schlolaut W, Hudson R, Rödel HG (2013) Impact of rearing management on health in domestic rabbits: A review. World Rabbit Sci 21:145–159. https://doi.org/10.4995/wrs.2013.1029

34. Hoy S, Verga M (2006) Welfare indicators. In: Martens L., Coudert P. (Eds.), Recent advances in Rabbit Sciences. ILVO: Melle, Belgium. pp 71–74

35. Bigler L, Oester H (1997) Untersuchung zum Einfluss des Lichtes in der Kaninchenmast. In: Arbeitstagung Dt. Vet. Med. Ges., Celle, Germany. pp 2011–216

36. Brummer H (1975) Trichophagie - eine Verhaltensstörung bei Kaninchen. Deut Tierärztl Woch 82:350–351

37. Seidel K (1936) Ergebnisse einer statistischen Auswertung von über 10.000 Kaninchensektionen. In: VI. Weltgeflügelkongress, Leipzig, Germany. pp 264–269

38. Kühn T (2003) Kokzidien des Kaninchens (Oryctolagus cuniculus) - Verlauf natürlicher Infektionen bei Boden- und Käfighaltung in einer Versuchstiereinheit. Thesis, University of Leipzig, Germany.

39. Ruis M (2006) Group housing of breeding does. In: Martens L., Coudert P. (Eds.), Recent advances in Rabbit Sciences. ILVO: Melle, Belgium. pp 99–105

40. Dal Bosco A, Castellini C, Mugnai C (2002) Rearing rabbits on a wire net floor or straw litter: behaviour, growth and meat qualitative traits. Livest Prod Sci 75:149–156. https://doi.org/10.1016/S0301-6226(01)00307-4

41. Abecia L, Fondevila M, Balcells J, McEwan NR (2007) The effect of lactating rabbit does on the development of the caecal microbial community in the pups they nurture. J Appl Microbiol 103:557–564

42. Rödel HG (2005) Winter feeding behaviour of European rabbits in a temperate zone habitat. Mamm Biol 70:300–306. https://doi.org/10.1016/j.mambio.2005.03.001

43. Rogers M, Arthur P, Soriguer C (1994) The rabbit in continental Europe. In: Thomson H., King C. (Eds.), The European Rabbit. History and Biology of a Successful Colonizer. Oxford University, Press: Oxford, UK. pp 22–63

44. Somers N, D'Haese B, Bossuyt B, Lens L, Hoffmann M (2008) Food quality affects diet preference of rabbits: Experimental evidence. Belg J Zool 138:170–176

45. Wolf P, Wenger A, Kamphues J (1997) Probleme der Rohfaserversorgung von Zwergkaninchen, Meerschweinchen und Chinchilla als Heimtiere. In: Arbeitstagung Dt. Vet. med. Ges., Celle, Germany. pp 154–165

46. Wolf P, Zumbrock B, Kamphues J (2005) Untersuchungen zu rassebedingten Einlüssen auf verschiedene Verdauungsprozesse sowie die Verdaulichkeit von Futtermitteln beim Kaninchen (Deutsche Riesen, Neuseeländer, Zwergkaninchen). In: Arbeitstagung Dt. Vet. Med. Ges., Celle, Germany. pp 186–194

47. Meo D, Bovera F, Marono S, Vella N, Nizza A (2007) Effect of feed restriction on performance and feed digestibility in rabbits. Ital J Anim Sci 6:765–767. https://doi.org/10.4081/ijas.2007.1s.765

48. Gidenne T (1993) Measurement of the rate of passage in restrictedfed rabbits: effect of dietary cell wall level on the transit of fibre particles of different sizes. Anim Feed Sci Technol 42:151–163

49. Scholaut W, Lange K (1990) Einfluß einer limitierten Futteraufname auf Wachstum und Futterverwertung beim Kaninchen. pp 118–124

50. Schlolaut W, Lange K, Schluter H (1978) Einfluss der Futterungsintensitat auf die Mastleistung und die Schlachtkorperqualitat beim Jungmastkaninchen. Zuchtungskunde 50:401–411

51. Schlolaut W, Lange K (1979) Kompensatorisches Wachstum bei Jungmastkaninchen. Zuchtungskunde 51:227–233

52. Duperray J, Eckenfelder B, Puybasset A, Richard A, Rouault M (2000) Interest of zinc bacitracin in the treatment and the prevention of the epizootic rabbit enterocolitis syndrome in growing rabbit. World Rabbit Sci 8:233–240

53. Boisot P, Duperray J, Guyonvarch A, Richard A, Licois D, Coudert P (2004) Evaluation of the effectiveness of soluble bacitracin (Bacivet S®) in drinking water compared to bacitracin in the feed (Albac®) during an experimental reproduction of epizootic rabbit enteropathy syndrome. In: Proc. 8th World Rabbit Congress, Puebla, Mexico. pp 457–462

54. Smith MV (2013) Textbook of rabbit medicine, 2nd ed. Elsevier Health Sciences, Oxford, UK

55. Dip R, Nemet Z, Schiessl B, Klein U, Strehlau G (2015) Efficacy and tolerability of early administration of valnemulin hydrochloride premix on epizootic rabbit enteropathy. Vet J 204:309–314. https://doi.org/10.1016/j.tvjl.2014.12.036

56. Cunha S, Mendes Â, Rego D, Meireles D, Fernandes R, Carvalho A, Costa PM da (2017) Effect of competitive exclusion in rabbits using an autochthonous probiotic. World Rabbit Sci 25:123–134. https://doi.org/10.4995/wrs.2017.4533

57. La Ragione RM, Narbad A, Gasson MJ, Woodward MJ (2004) In vivo characterization of Lactobacillus johnsonii FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. Lett Appl Microbiol 38:197–205. https://doi.org/10.1111/j.1472-765X.2004.01474.x

58. Philippeau C, Respondek F, Julliand V (2010) In vitro effects of fructo-oligosaccharides on bacterial concentration and fermentation profiles in veal calf ileal contents. Anim Feed Sci Technol 162:83–90

59. Chu GM, Lee SJ, Jeong HS, Lee SS (2011) Efficacy of probiotics from anaerobic microflora with prebiotics on growth performance and noxious gas emission in growing pigs. Anim Sci J 82:282–290

60. Salyers AA, Gupta A, Wang Y (2004) Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol 12:412–416

61. Mathur S, Singh R (2005) Antibiotic resistance in food lactic acid bacteria—a review. Int J Food Microbiol 105:281–295

62. Galyean ML, Eng KS (1998) Application of research findings and summary of research needs: Bud Britton Memorial Symposium on Metabolic Disorders of Feedlot Cattle. J Anim Sci 76:323. https://doi.org/10.2527/1998.761323x

63. Schneitz C (2005) Competitive exclusion in poultry—30 years of research. Food Control 16:657–667. https://doi.org/10.1016/j.foodcont.2004.06.002