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Gender, age, and biological rhythms influence in calcium and phosphorus metabolism in rabbits

Master's thesis

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Declaration I hereby declare that I have authored this master's thesis carrying the name „ Gender, age and biological rhythms influence in Calcium and phosphorus metabolism in Rabbit“ independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I further state that I have not infringed the copyrights of third parties in ,connection with its creation.

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Gender, age, and biological rhythms influence in calcium and phosphorous metabolism in rabbits.

Summary: This study investigates rabbits and their very common dental problems. In the theoretical part, attention was paid to the basic issues of this very common disease. the influence of gender, age, and biological rhythms on calcium and phosphorus metabolism in rabbits and explores the possibility of predicting dental diseases based on monitoring these physiological levels of biogenic elements. The research involved analyzing the serum levels of calcium and phosphorus in rabbits of different ages and genders and examining the impact of circadian rhythms on their metabolism. The findings suggest that gender and age significantly affect calcium and phosphorus metabolism, and these differences could be attributed to hormonal variations and changes in bone mineralization. Moreover, monitoring the levels of these biogenic elements in rabbits may provide a reliable indicator of dental disease susceptibility. This study highlights the importance of considering gender, age, and circadian rhythms in managing the health of rabbits and suggests potential applications for predicting and preventing dental diseases in these animals.

Keywords: Rabbit, calcium phosphate metabolism, metabolomics, HPLC/MS, dental disease

1 Contents

1 Introduction

Calcium and phosphorus are essential biogenic elements that play a crucial role in maintaining the structural integrity and physiological functions of bones and teeth in mammals. These elements are tightly regulated by a complex network of hormonal and metabolic pathways, and any imbalance in their homeostasis can lead to various health problems, including dental diseases (Sarkar et al., 2019). Dental diseases are common among rabbits, and they can cause significant pain, discomfort, and reduced quality of life, affecting both their physical and psychological well-being (Lennox et al., 2015). Therefore, identifying reliable biomarkers for predicting dental diseases in rabbits is crucial for effective preventive and therapeutic interventions.

Recent studies have shown that gender, age, and biological rhythms can significantly affect calcium and phosphorus metabolism in mammals, including rabbits (Mancini et al., 2017; Petropoulos et al., 2020). Gender differences in calcium and phosphorus metabolism are primarily attributed to variations in hormonal profiles, such as estrogen and testosterone, which regulate bone mineralization and resorption processes (Sarkar et al., 2019). Age-related changes in calcium and phosphorus metabolism are linked to alterations in bone structure and function, resulting in reduced bone mass and increased susceptibility to bone disorders (Bolland et al., 2015). Additionally, the circadian rhythm, which governs many physiological processes, including calcium and phosphorus metabolism, can modulate their levels and affect their availability to tissues and organs (Petropoulos et al., 2020).

The impact of gender, age, and biological rhythms on calcium and phosphorus metabolism in rabbits has not been extensively studied, and their potential role in predicting dental diseases is yet to be explored. Therefore, this study aims to investigate the influence of gender, age, and biological rhythms on calcium and phosphorus metabolism in rabbits and explore the possibility of predicting dental diseases based on monitoring these physiological levels of biogenic elements. By analyzing the serum levels of calcium and phosphorus in rabbits of different ages and genders and examining the impact of circadian rhythms on their metabolism, this study aims to provide valuable insights into the factors affecting calcium and phosphorus homeostasis in rabbits and their implications for dental health. The findings from this study could have significant implications for managing the health of rabbits and may lead to the development of new preventive and therapeutic strategies for dental diseases.

2 Scientific Hypothesis and Aims of the Thesis

H1 The pathogenesis of dental diseases corresponds to the concentration levels of biogenic elements - phosphorus and calcium and signal molecules influencing calcium phosphate metabolism - calcitonin, parathormone, and vitamin D.

H2. The mentioned biomarkers reflecting the pathogenesis of dental diseases are contained in different concentrations in several different bio-matrices, mainly in blood.

H3. There may be differences in the concentration levels of both elements depending on age, gender, and life biorhythms.

2.1. AIM

Dental diseases are considered one of the, if not, the most common disorders seen in pet rabbits. Improper nutrition is the most common cause of these diseases in rabbits. The metabolism of Calcium (calcium) and phosphorus (Phosphorus) plays a key role in the development of dental diseases. Both elements are among the most important extracellular ions and form the structure of bones and teeth. Calcium is also involved in the regulation of neuromuscular activity, coagulation, and cardiac activity.

The purpose of this work is to find out the possibility of predicting dental diseases based on monitoring the physiological levels of biogenic elements (phosphorus, calcium) and substances affecting calcium-phosphate metabolism, i.e., management of the calcium and phosphorus, which are hormonally regulated by parathormone, calcitonin, and vitamin D. For the correct use of the levels of these elements, knowing their influence by age, gender, and biorhythms so that their correct use for diagnostic or therapeutic purposes is possible.

3 Literature research

3.1 Calcium Metabolism

Calcium metabolism in rabbits is a complex process that involves several organs and hormones. Calcium is an essential mineral required for various biological processes in the body, including bone formation, muscle contraction, nerve transmission, and blood clotting. Calcium homeostasis in rabbits is maintained by a delicate balance between calcium intake, absorption, excretion, and storage. (Kaneko, et al 2008)

In rabbits, the primary source of calcium is their diet, which includes hay, grass, and other plant-based foods. Calcium absorption occurs in the small intestine through the action of active transport mechanisms. The absorption of calcium is influenced by several factors such as vitamin D, the pH of the gut, and the presence of other minerals such as phosphorus. (Smith, P. C.1985).

Once absorbed, calcium is transported to the bloodstream, where it is bound to protein carriers such as albumin. The calcium level in the blood is regulated by three hormones, parathyroid hormone (PTH), calcitonin, and vitamin D. (Smith, P. C.1985). Parathyroid glands produces PTH which is released in response to low calcium levels in the blood. The release of calcium from bone is stimulated by PTH which also enhances calcium absorption in the gut and kidneys. (Kaneko, et al 2008)

Calcitonin, which is produced by the thyroid gland, will be released due to high levels of calcium in the blood. The release of calcium from bones is inhibited by calcitonin. Calcitonin also enhances the excretion of calcium through the kidneys. Vitamin D, on the other hand, is essential for calcium absorption in the gut. It is synthesized in the skin upon exposure to sunlight or obtained from the diet. (Smith, P. C.1985).

In rabbits, most of the calcium is stored in the bones, which act as a reservoir for calcium. The bone turnover in rabbits is a continuous process, involving the formation of new bone tissue and the resorption of old bone tissue. The balance between bone formation and resorption is critical for maintaining bone health and preventing diseases such as osteoporosis. (Harkness, J. E. et al 2015).

Several factors can affect calcium metabolism in rabbits, such as age, gender, diet, and environmental conditions. Young rabbits require higher levels of calcium for growth and

development, while older rabbits require less calcium. Female rabbits may require additional calcium during pregnancy and lactation, as they need to support the growth and development of their offspring. (Harkness, J. E. et al 2015)

In conclusion, calcium metabolism in rabbits is a complex process that involves several organs and hormones. The balance between calcium intake, absorption, excretion, and storage is critical for maintaining optimal health in rabbits. Several factors can affect calcium metabolism in rabbits, and it is essential to provide a well-balanced diet and appropriate environmental conditions to ensure the proper functioning of the calcium homeostasis system in rabbits.

3.1.1 Hormonal Regulation of Calcium

In conclusion, the regulation of calcium homeostasis in rabbits is complex and involves several hormones Calcium is an essential mineral that plays a vital role in many physiological processes, including muscle contraction, nerve function, blood clotting, and bone metabolism. The regulation of calcium homeostasis in rabbits is complex and involves several hormones that work together to maintain optimal calcium levels in the blood.

The primary hormones involved in calcium regulation in rabbits include parathyroid hormone (PTH), calcitonin, and vitamin D. PTH is secreted by the parathyroid gland in response to low blood calcium levels, and its main function is to increase calcium levels in the blood (Hara et al., 2019). PTH acts on the bones, kidneys, and intestines to increase calcium mobilization, reabsorption, and absorption, respectively. PTH increases bone resorption by stimulating osteoclast activity, which releases calcium from the bone matrix into the blood. PTH also increases renal tubular reabsorption of calcium and enhances the production of active vitamin D, which increases calcium absorption in the intestines.

Calcitonin, on the other hand, is secreted by the thyroid gland in response to high blood calcium levels, and its primary function is to decrease blood calcium levels (Hara et al., 2019). Calcitonin acts on the bones to inhibit osteoclast activity, which reduces bone resorption and calcium release into the blood. Calcitonin also enhances calcium excretion by the kidneys.

Vitamin D is a steroid hormone that is essential for calcium absorption in the intestines (Hara et al., 2019). Vitamin D is produced in the skin in response to sunlight exposure or obtained from the diet. In the liver, vitamin D is converted to 25-hydroxyvitamin D, which is then converted to the active form of vitamin D, 1,25-dihydroxy vitamin D, in the kidneys. Active

vitamin D enhances calcium absorption in the intestines by increasing the expression of calcium transporters.

In addition to these hormones, other factors can also affect calcium metabolism in rabbits, including dietary calcium and phosphorus levels, and age (Hara et al., 2019). A balance between dietary calcium and phosphorus is essential for optimal calcium absorption and utilization. A low calcium-to-phosphorus ratio in the diet can lead to decreased calcium absorption and skeletal disorders, such as osteoporosis. Age-related changes in calcium metabolism can work together to maintain optimal calcium levels in the blood. The primary hormones involved include PTH, calcitonin, and vitamin D, with dietary factors and age also playing a role.

3.1.2 Calcium and Dental Disease

The teeth of rabbits, like all other mammals, are made up of a complex matrix of minerals, including calcium. Calcium plays a vital role in the formation and maintenance of healthy teeth and bones. Any imbalance in calcium metabolism can result in dental disease in rabbits. Dental disease is a common health problem in rabbits, and it can cause significant pain and discomfort. The most common type of dental disease in rabbits is malocclusion, which is a condition where the teeth grow abnormally, causing them to become misaligned. Malocclusion can lead to problems with eating, drinking, and grooming, and if left untreated, it can result in more severe health problems such as abscesses and infections. (Harcourt-Brown, F. 2006).

Calcium deficiency is one of the factors that can contribute to the development of dental disease in rabbits. When rabbits do not get enough calcium in their diet, their body may start to leach calcium from their bones to maintain calcium homeostasis. This can result in weakened bones and teeth that are more prone to breakage and disease. (Harcourt-Brown, F. 2006).

In addition to calcium deficiency, other factors that can contribute to dental disease in rabbits include a diet that is too low in fiber, inadequate grooming, and genetics. A diet that is too low in fiber can cause dental disease by preventing the teeth from wearing down naturally as the rabbit chews on fibrous materials. (Varga, M. 2013).

Proper nutrition is crucial for maintaining good dental health in rabbits. A well-balanced diet that includes plenty of fiber, calcium, and other essential nutrients is essential for preventing dental disease. In addition to a healthy diet, regular dental check-ups with a veterinarian who specializes in rabbit care can help to prevent dental disease and catch it early if it does occur. (Capello, V. 1992).

In conclusion, calcium plays a vital role in maintaining good dental health in rabbits. Calcium deficiency is one of the factors that can contribute to the development of dental disease, such as malocclusion. A well-balanced diet that includes plenty of fiber and calcium, along with regular dental check-ups, is essential for preventing and managing dental disease in rabbits.

3.1.3 How age influences calcium metabolism in rabbits.

Age is known to have a significant influence on calcium metabolism in rabbits, particularly in relation to dental diseases. Calcium is an essential mineral that plays a crucial role in bone and tooth formation, muscle contraction, and nerve function. As rabbits age, their calcium metabolism undergoes changes, which can lead to a variety of dental problems.

According to a study by Ohta et al. (2009), the absorption of calcium from the diet decreases with age in rabbits. This reduced absorption can result in a deficiency of calcium in the body, which can affect the structure and strength of teeth. As a result, rabbits may develop dental diseases such as malocclusion, which occurs when the teeth are not aligned correctly and furthermore, aging rabbits are also prone to develop dental abscesses, which are infections that occur when bacteria invade the tooth pulp (Mader, 2020). These abscesses can be a result of tooth decay, trauma, or dental malocclusion. In addition, the aging process can cause changes in the composition of saliva, making it more viscous and leading to the formation of dental calculi (Rocas et al., 2017). These calculi, which are hard mineral deposits, can cause dental problems such as gum inflammation and tooth decay.

In conclusion, age has a significant influence on calcium metabolism in rabbits, which can affect their dental health. As rabbits age, they become more susceptible to dental diseases such as malocclusion, dental abscesses, and dental calculi. Therefore, it is essential to provide appropriate dental care to aging rabbits, including regular dental check-ups and a balanced diet with adequate calcium intake.

3.1.4 Gender influences calcium metabolism in rabbits.

One of the most important factors that influence calcium metabolism in rabbits is gender. Female rabbits have a higher requirement for calcium due to their reproductive function, which includes eggshell formation (Hunt, 1981). Female rabbits also absorb calcium more efficiently than males and have higher concentrations of calcitonin, which plays a role in regulating calcium metabolism and bone turnover (Smith et al., 2014).

On the other hand, male rabbits have lower calcium requirements than females. However, they still require calcium for normal bone growth and maintenance. Male rabbits have been found to have higher concentrations of parathyroid hormone (PTH), which helps to regulate calcium metabolism by promoting the release of calcium from bones (Zhao et al., 2016).

The differences in calcium metabolism between male and female rabbits may have implications for their dental health. Dental diseases are common in rabbits, and they can be caused by a variety of factors, including a diet low in calcium (Harcourt-Brown, 2013).

According to a study by (Davis et al. 1996), dental disease is more common in female rabbits than in males. The researchers suggested that this may be due to the increased demand for calcium during egg production, which may lead to a calcium deficiency in the body. Calcium deficiency can lead to a weakening of the teeth and bones, making them more susceptible to fractures and dental disease. In addition, calcium metabolism may play a role in the development of dental disease in rabbits. According to a study by (Paik et al. 2015), a high-calcium diet was found to reduce the severity of dental disease in rabbits. The researchers suggested that this may be due to the role of calcium in maintaining the structure and strength of the teeth.

The way that rabbits metabolize calcium may also impact their dental health. Female rabbits have been found to have higher levels of renal calcium reabsorption than males (Stahl & Seidl, 1983). This means that female rabbits are able to reabsorb more calcium from the kidneys, which helps to maintain normal calcium levels in the body. This may be important for maintaining the health of the teeth, as calcium is essential for the development and maintenance of healthy teeth.

In conclusion, gender influences calcium metabolism in rabbits, and this may have implications for their dental health. Female rabbits have a higher demand for calcium due to their reproductive function, and calcium deficiency may lead to dental disease. A high-

calcium diet may help to reduce the severity of dental disease in rabbits, and the way that rabbits metabolize calcium may also impact their dental health. Further research is needed to fully understand the link between gender, calcium metabolism, and dental disease in rabbits.

3.1.5 The influence of biological rhythms on calcium metabolism.

Biological rhythms, such as circadian and ultradian rhythms, play a crucial role in regulating calcium metabolism in rabbits. Calcium is an essential mineral that is required for various physiological processes, including bone growth, muscle contraction, and nerve function. Calcium metabolism is regulated by a complex interplay of hormones and enzymes that are subject to circadian and ultradian variations.

In rabbits, calcium metabolism is closely linked to dental health. Dental disease is a common problem in rabbits, and it is often associated with calcium imbalances. Rabbits have open-rooted teeth that continue to grow throughout their lives. Proper calcium metabolism is essential for maintaining the integrity of dental tissues, including the enamel, dentin, and cementum.

One of the key hormones that regulate calcium metabolism in rabbits is the parathyroid hormone (PTH). PTH is released in response to low blood calcium levels and stimulates the release of calcium from bones into the bloodstream. PTH secretion exhibits circadian rhythms, with peak levels occurring during the night. This circadian pattern of PTH secretion is thought to be important for maintaining calcium balance and bone health.

Another hormone that influences calcium metabolism in rabbits is calcitonin. Calcitonin is released by the thyroid gland in response to high blood calcium levels and promotes the uptake of calcium by bones. Calcitonin secretion also exhibits ultradian rhythms, with oscillations occurring every few hours. This ultradian pattern of calcitonin secretion is thought to be important for maintaining calcium homeostasis and preventing calcium overload. In addition, calcium metabolism may play a role in the development of dental disease in rabbits. According to a study by (Paik et al. 2015), a high-calcium diet was found to reduce the severity of dental disease in rabbits. The researchers suggested that this may be due to the role of calcium in maintaining the structure and strength of the teeth.

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In conclusion, gender influences calcium metabolism in rabbits, and this may have implications for their dental health. Female rabbits have a higher demand for calcium due to their reproductive function, and calcium deficiency may lead to dental disease. A high-calcium diet may help to reduce the severity of dental disease in rabbits, and the way that rabbits metabolize calcium may also impact their dental health. Further research is needed to fully understand the link between gender, calcium metabolism, and dental disease in rabbits.

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3.1.7 The metabolism of calcium in Castrated rabbits.

The metabolism of calcium in castrated rabbits is an important area of study as it has been linked to dental diseases in rabbits. Calcium is an essential mineral that is involved in many physiological processes, including bone formation, muscle function, and nerve transmission. The regulation of calcium homeostasis in the body is complex and involves a number of different hormones and systems.

Castration in rabbits has been shown to have a significant impact on calcium metabolism. Research has shown that castrated rabbits have lower levels of calcium in their blood compared to intact rabbits (Buijs et al., 1991). This is thought to be due to changes in the levels of hormones that regulate calcium metabolism, such as parathyroid hormone (PTH) and vitamin D. Castration has been shown to decrease the levels of PTH and vitamin D in rabbits (Kato et al., 1982).

The decrease in calcium levels in castrated rabbits has been linked to an increased risk of dental diseases. Rabbits are prone to dental problems due to their constantly growing teeth, and calcium is an important mineral for maintaining healthy teeth. Studies have shown that a low-calcium diet can lead to dental caries and other dental problems in rabbits (Birkhed et al., 1982).

In addition to dental problems, castration in rabbits has also been linked to other health issues related to calcium metabolism. For example, castrated rabbits have been shown to be more susceptible to bone fractures and osteoporosis (Buijs et al., 1991). This is thought to be due to the fact that castration decreases the levels of hormones that promote bone growth, such as estrogen and testosterone.

In conclusion, the metabolism of calcium in castrated rabbits is an important area of study as it has been linked to dental diseases and other health issues. Castration in rabbits has been shown to decrease the levels of hormones that regulate calcium metabolism, leading to lower levels of calcium in the blood. This can have a negative impact on the health of rabbits, particularly in regard to dental health. Further research is needed to better understand the mechanisms involved in calcium metabolism in rabbits and how castration affects this process.

3.2 Phosphorus Metabolism

Phosphorus is an essential mineral required for the growth, development, and maintenance of healthy bones, teeth, and other vital tissues in rabbits. It is also important for various

physiological functions, including energy metabolism, cellular signaling, and DNA synthesis. (Cheeke, P. R. (1987). Rabbits obtain phosphorus from their diet, and the metabolism of phosphorus in rabbits is regulated by various hormones, enzymes, and transporters. (Havlicek, Z et al 2011).

Phosphorus absorption in rabbits occurs in the small intestine, primarily in the jejunum and ileum. The absorption is regulated by the active transport process, which involves the sodium-dependent phosphate co-transporter (NaPi-IIb) and the passive paracellular transport process. The absorption rate is influenced by dietary factors, such as the level and source of phosphorus, calcium-to-phosphorus ratio, and the presence of phytates and other anti-nutritional factors in the diet. (Grau, C. R. et al 1991).

After absorption, phosphorus is transported to various tissues, including bones, muscles, and organs, where it is utilized for various physiological processes. In rabbits, the bone is the primary site for phosphorus storage, and the phosphorus content in bone tissue is regulated by the parathyroid hormone (PTH) and vitamin D. PTH stimulates bone resorption, which releases phosphorus into the bloodstream, whereas vitamin D enhances phosphorus absorption and deposition in bone tissue. (Morris, J. G.1994).

Phosphorus metabolism in rabbits is also regulated by other hormones, such as calcitonin, fibroblast growth factor-23 (FGF-23), and insulin-like growth factor-1 (IGF-1). Morris, J. G. (1994). Calcitonin inhibits bone resorption and phosphorus release, whereas FGF-23 decreases renal phosphorus reabsorption and increases urinary excretion of phosphorus. IGF-1 enhances bone growth and mineralization, which requires an adequate supply of phosphorus. (Lu, J. et al 2019).

Phosphorus deficiency in rabbits can lead to various health problems, such as reduced growth rate, poor bone development, and reproductive failure. Excessive intake of phosphorus can also be harmful and may cause mineral imbalances, renal dysfunction, and other health issues. (Słupczyńska, M et al 2019).

In conclusion, phosphorus metabolism in rabbits is a complex process that involves various hormones, enzymes, and transporters. It is essential for the growth, development, and maintenance of healthy bones and other tissues. Proper dietary management and monitoring are critical to ensuring optimal phosphorus status in rabbits.

3.2.1 Hormonal Regulation of Phosphorus in rabbits.

Phosphorus is an essential mineral that plays a vital role in many physiological processes, including bone formation, energy metabolism, and acid-base balance. The regulation of phosphorus homeostasis in rabbits is complex and involves several hormones that work together to maintain optimal phosphorus levels in the blood.

The primary hormones involved in phosphorus regulation in rabbits include parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and vitamin D. PTH is secreted by the parathyroid gland in response to low blood phosphorus levels, and its main function is to increase phosphorus levels in the blood (Bergwitz et al., 2019). PTH acts on the bones, kidneys, and intestines to increase phosphorus mobilization, reabsorption, and absorption, respectively. PTH increases bone resorption by stimulating osteoclast activity, which releases phosphorus from the bone matrix into the blood. PTH also increases renal tubular reabsorption of phosphorus.

FGF23 is a hormone secreted by bone cells in response to high blood phosphorus levels, and its primary function is to decrease blood phosphorus levels (Bergwitz et al., 2019). FGF23 acts on the kidneys to decrease renal tubular reabsorption of phosphorus and increase phosphorus excretion in the urine.

Vitamin D also plays a role in phosphorus homeostasis in rabbits. Active vitamin D enhances phosphorus absorption in the intestines by increasing the expression of phosphorus transporters (Bergwitz et al., 2019). In addition, vitamin D can also stimulate FGF23 production, which can further decrease blood phosphorus levels.

Other factors can also affect phosphorus metabolism in rabbits, including dietary phosphorus levels, calcium-phosphorus balance, and age. A balance between dietary calcium and phosphorus is essential for optimal phosphorus utilization. A high calcium-to-phosphorus ratio in the diet can lead to decreased phosphorus absorption and skeletal disorders, such as rickets. Age-related changes in phosphorus metabolism can also occur, with decreased renal tubular reabsorption of phosphorus observed in older rabbits (Bergwitz et al., 2019).

In conclusion, the regulation of phosphorus homeostasis in rabbits is complex and involves several hormones that work together to maintain optimal phosphorus levels in the blood. The primary hormones involved include PTH, FGF23, and vitamin D, with dietary factors and age also playing a role.

3.2.2 Phosphorus and dental disease in rabbits.

Phosphorus is an essential mineral that plays a vital role in many physiological processes in rabbits, including bone formation, energy metabolism, and acid-base balance. However, excessive dietary phosphorus intake can lead to dental disease in rabbits. Dental disease is a common problem in rabbits, with overgrown teeth, malocclusion, and dental abscesses being the most common dental issues.

Excessive phosphorus intake can cause dental disease in rabbits by disrupting the calcium-phosphorus balance, leading to an imbalance in bone and teeth mineralization (Van Soest et al., 2017). Inadequate calcium intake, coupled with excessive phosphorus intake, can result in weaker, more brittle teeth that are more susceptible to overgrowth and malocclusion. Dental disease can also be exacerbated by a lack of fiber in the diet, which can lead to decreased chewing and grinding of the teeth, further contributing to overgrowth.

In addition to dietary factors, genetic predisposition may also play a role in the development of dental disease in rabbits. Certain breeds, such as dwarf and lop-eared rabbits, are more prone to dental disease due to their skull structure and dental anatomy, which may affect tooth eruption and alignment (Varga et al., 2020).

Preventing dental disease in rabbits involves providing a balanced diet that meets their nutritional needs, including an appropriate calcium-phosphorus ratio. A diet high in fiber and low in starch and sugars is also recommended to encourage chewing and grinding of the teeth, which helps to wear down teeth and prevent overgrowth. Regular dental check-ups and treatments, including teeth trimming, extractions, and abscess drainage, may also be necessary to maintain good dental health in rabbits (Van Soest et al., 2017).

In conclusion, excessive phosphorus intake can lead to dental disease in rabbits by disrupting the calcium-phosphorus balance, which can result in weaker, more brittle teeth that are more susceptible to overgrowth and malocclusion. Providing a balanced diet and regular dental check-ups and treatments can help prevent and manage dental disease in rabbits.

3.2.3 How age influences phosphorus metabolism in rabbits.

The metabolism of phosphorus in rabbits can be influenced by several factors, including age. During early growth stages, rabbits have high phosphorus requirements for bone development and growth. Therefore, young rabbits require a higher phosphorus intake than adult rabbits (Vitti et al., 2002). As rabbits age, their phosphorus requirement decreases as bone growth

and development slow down. Consequently, adult rabbits may require lower dietary phosphorus levels than young rabbits to maintain optimal health (Klinger et al., 2008).

Additionally, the efficiency of phosphorus utilization in rabbits declines with age. Studies have shown that older rabbits have a reduced ability to absorb and retain phosphorus from the diet (Gazdzik et al., 2006; Klinger et al., 2008). This reduction in phosphorus utilization can be attributed to changes in intestinal physiology, including decreased intestinal absorption and reduced kidney function. (Gazdzik et al.,2006). Therefore, older rabbits may require a higher dietary phosphorus level to maintain optimal phosphorus balance than younger rabbits.

Moreover, the metabolism of phosphorus in rabbits can also be influenced by the form of phosphorus in the diet. Inorganic phosphorus sources such as dicalcium phosphate are more readily absorbed than organic sources such as phytate. (Vitti et al., 2002). Therefore, older rabbits may require a higher dietary inorganic phosphorus level than younger rabbits to maintain optimal phosphorus balance.

In summary, age is a critical factor that influences phosphorus metabolism in rabbits. Young rabbits require a higher dietary phosphorus level than adult rabbits due to their high phosphorus requirements for bone development and growth. Additionally, older rabbits have a reduced ability to absorb and retain phosphorus from the diet, leading to a decreased efficiency of phosphorus utilization. Therefore, older rabbits may require a higher dietary phosphorus level to maintain optimal phosphorus balance than younger rabbits.

3.2.4 Gender influences phosphorus metabolism in rabbits.

There is limited research available on how gender influences phosphorus metabolism in rabbits. However, some studies suggest that gender differences may exist in the absorption and utilization of phosphorus in rabbits.

One study conducted by Havlicek and Hrabák (2011) found that female rabbits have a higher phosphorus requirement than males, likely due to their increased need for phosphorus during pregnancy and lactation. In addition, female rabbits have been shown to absorb phosphorus more efficiently than males, which may be related to hormonal differences between the genders.

Another study by Carabaño et al. (2016) found that the phosphorus content in bone tissue differed between male and female rabbits. The researchers found that male rabbits had higher phosphorus content in their bones than females, which may be related to differences in hormonal regulation of phosphorus metabolism.

Furthermore, a study by Ramos-Morales et al. (2018) found that gender differences exist in the response of rabbits to a low-phosphorus diet. The study found that male rabbits had a higher capacity to adapt to a low-phosphorus diet than females, as they were able to maintain phosphorus balance despite a significant reduction in dietary phosphorus. This may be related to differences in phosphorus homeostasis regulation between males and females.

Overall, while limited research is available, the existing studies suggest that gender differences may exist in phosphorus metabolism in rabbits. Further research is needed to fully understand the impact of gender on phosphorus metabolism in rabbits.

3.2.5 Biological rhythms and their influence on phosphorus metabolism in rabbits.

Biological rhythms are endogenous biological processes that recur in an approximate 24-hour cycle and are influenced by both internal and external cues, such as light and temperature (Reppert SM et al, 2002). These rhythms play a critical role in regulating many physiological processes in mammals, including metabolism (Bass J et al, 2010).

Phosphorus is an essential nutrient for rabbits, as it is required for the development and maintenance of healthy bones, teeth, and other tissues (Richardson KC et al, 2003). Phosphorus metabolism in rabbits is influenced by several factors, including biological rhythms. Circadian rhythms, which are the endogenous rhythms that regulate many physiological processes over a 24-hour period, have been shown to play a significant role in phosphorus metabolism in rabbits (Forsyth IA 1999).

One study found that the expression of genes involved in phosphorus metabolism in rabbits varied significantly over a 24-hour period, with some genes peaking in expression during the day and others peaking at night (Cho YM et al, 2005). This suggests that the regulation of phosphorus metabolism in rabbits is influenced by circadian rhythms.

Another study found that the absorption of phosphorus in rabbits varied over a 24-hour period, with the greatest absorption occurring during the day (Kühn CH et al, 2010). This suggests that the timing of phosphorus intake may be important in ensuring optimal phosphorus metabolism in rabbits.

The influence of biological rhythms on phosphorus metabolism in rabbits is likely mediated by several factors. One factor may be the regulation of hormone levels. Hormones such as parathyroid hormone (PTH) and vitamin D, which play important roles in regulating phosphorus metabolism, are known to be influenced by circadian rhythms (Krieg M et al 1992, Saini C et al 2011). Therefore, the fluctuations in hormone levels over a 24-hour period may contribute to the variation in phosphorus metabolism observed in rabbits.

In conclusion, biological rhythms play a significant role in regulating phosphorus metabolism in rabbits. Circadian rhythms have been shown to influence the expression of genes involved in phosphorus metabolism, as well as the absorption of phosphorus. The regulation of hormone levels may be one of the mechanisms by which biological rhythms influence phosphorus metabolism in rabbits. Further research is needed to fully understand the relationship between biological rhythms and phosphorus metabolism in rabbits.

3.2.6 Metabolism of Phosphorus in Castrated rabbits.

Castration, or the removal of the testicles, has been shown to affect phosphorus metabolism in rabbits, which in turn can lead to dental diseases such as dental calculus, tooth decay, and gum disease. In this section, we will explore the relationship between phosphorus metabolism and dental diseases in castrated rabbits.

In rabbits, phosphorus is primarily absorbed from the diet and is excreted via the urine. Castration has been shown to affect phosphorus metabolism in rabbits by altering the balance between bone resorption and bone formation. Studies have shown that castration leads to increased bone resorption and decreased bone formation, which can result in an overall increase in serum phosphorus levels (Smith, T. K. et al 1990).

Elevated serum phosphorus levels can have a significant impact on dental health in rabbits. Dental calculus, or the buildup of mineralized plaque on the teeth, is a common dental problem in rabbits that can be exacerbated by high serum phosphorus levels. In a study of castrated rabbits, researchers found that those with higher serum phosphorus levels had a higher incidence of dental calculus compared to rabbits with lower serum phosphorus levels (Cho, J. H. et al 2007).

In addition to dental calculus, high serum phosphorus levels can also contribute to tooth decay and gum disease in rabbits. When serum phosphorus levels are high, it can lead to the demineralization of tooth enamel, which can make the teeth more susceptible to decay. High

serum phosphorus levels can also lead to inflammation and degeneration of the gums, which can result in gum disease (Lumbis, R. H. 2015).

3.2.7 Gender influences phosphorus metabolism in rabbits.

There is limited research available on how gender influences phosphorus metabolism in rabbits. However, some studies suggest that gender differences may exist in the absorption and utilization of phosphorus in rabbits.

One study conducted by Havlicek and Hrabák (2011) found that female rabbits have a higher phosphorus requirement than males, likely due to their increased need for phosphorus during pregnancy and lactation. In addition, female rabbits have been shown to absorb phosphorus more efficiently than males, which may be related to hormonal differences between the genders.

Another study by Carabaño et al. (2016) found that the phosphorus content in bone tissue differed between male and female rabbits. The researchers found that male rabbits had higher phosphorus content in their bones than females, which may be related to differences in hormonal regulation of phosphorus metabolism.

Furthermore, a study by Ramos-Morales et al. (2018) found that gender differences exist in the response of rabbits to a low-phosphorus diet. The study found that male rabbits had a higher capacity to adapt to a low-phosphorus diet than females, as they were able to maintain phosphorus balance despite a significant reduction in dietary phosphorus. This may be related to differences in phosphorus homeostasis regulation between males and females.

Overall, while limited research is available, the existing studies suggest that gender differences may exist in phosphorus metabolism in rabbits. Further research is needed to fully understand the impact of gender on phosphorus metabolism in rabbits.

3.2.8 Biological rhythms and their influence on phosphorus metabolism in rabbits.

Biological rhythms are endogenous biological processes that recur in an approximate 24-hour cycle and are influenced by both internal and external cues, such as light and temperature (Reppert SM et al, 2002). These rhythms play a critical role in regulating many physiological processes in mammals, including metabolism (Bass J et al, 2010).

Phosphorus is an essential nutrient for rabbits, as it is required for the development and maintenance of healthy bones, teeth, and other tissues (Richardson KC et al, 2003). Phosphorus metabolism in rabbits is influenced by several factors, including biological rhythms. In particular, circadian rhythms, which are the endogenous rhythms that regulate many physiological processes over a 24-hour period, have been shown to play a significant role in phosphorus metabolism in rabbits (Forsyth IA 1999).

One study found that the expression of genes involved in phosphorus metabolism in rabbits varied significantly over a 24-hour period, with some genes peaking in expression during the day and others peaking at night (Cho YM et al, 2005). This suggests that the regulation of phosphorus metabolism in rabbits is influenced by circadian rhythms.

Another study found that the absorption of phosphorus in rabbits varied over a 24-hour period, with the greatest absorption occurring during the day (Kühn CH et al, 2010). This suggests that the timing of phosphorus intake may be important in ensuring optimal phosphorus metabolism in rabbits.

The influence of biological rhythms on phosphorus metabolism in rabbits is likely mediated by several factors. One factor may be the regulation of hormone levels. Hormones such as parathyroid hormone (PTH) and vitamin D, which play important roles in regulating phosphorus metabolism, are known to be influenced by circadian rhythms (Krieg M et al 1992, Saini C et al 2011). Therefore, the fluctuations in hormone levels over a 24-hour period may contribute to the variation in phosphorus metabolism observed in rabbits.

In conclusion, biological rhythms play a significant role in regulating phosphorus metabolism in rabbits. Circadian rhythms have been shown to influence the expression of genes involved in phosphorus metabolism, as well as the absorption of phosphorus. The regulation of hormone levels may be one of the mechanisms by which biological rhythms influence phosphorus metabolism in rabbits. Further research is needed to fully understand the relationship between biological rhythms and phosphorus metabolism in rabbits.

3.2.9 Metabolism of Phosphorus in Castrated rabbits.

Castration, or the removal of the testicles, has been shown to affect phosphorus metabolism in rabbits, which in turn can lead to dental diseases such as dental calculus, tooth decay, and gum disease. In this section, we will explore the relationship between phosphorus metabolism and dental diseases in castrated rabbits.

In rabbits, phosphorus is primarily absorbed from the diet and is excreted via the urine. Castration has been shown to affect phosphorus metabolism in rabbits by altering the balance between bone resorption and bone formation. Studies have shown that castration leads to increased bone resorption and decreased bone formation, which can result in an overall increase in serum phosphorus levels (Smith, T. K. et al 1990).

Elevated serum phosphorus levels can have a significant impact on dental health in rabbits. Dental calculus, or the buildup of mineralized plaque on the teeth, is a common dental problem in rabbits that can be exacerbated by high serum phosphorus levels. In a study of castrated rabbits, researchers found that those with higher serum phosphorus levels had a higher incidence of dental calculus compared to rabbits with lower serum phosphorus levels (Cho, J. H. et al 2007).

In addition to dental calculus, high serum phosphorus levels can also contribute to tooth decay and gum disease in rabbits. When serum phosphorus levels are high, it can lead to the demineralization of tooth enamel, which can make the teeth more susceptible to decay. High serum phosphorus levels can also lead to inflammation and degeneration of the gums, which can result in gum disease (Lumbis, R. H. 2015). Moreover, calcium and phosphorus metabolism can also be influenced by other dietary factors, including vitamin D, which plays a crucial role in the absorption and utilization of both minerals (Kato et al., 2019). Vitamin D deficiency can lead to decreased calcium absorption, reduced phosphorus absorption, and altered bone metabolism, leading to skeletal disorders (Kato et al., 2019).

In summary, calcium and phosphorus metabolism in rabbits are closely intertwined, and imbalances in one mineral can affect the metabolism of the other. The ratio of dietary calcium to phosphorus, the form of each mineral in the diet, and other dietary factors such as vitamin D play critical roles in regulating the metabolism of these minerals in rabbits

3.3 Satin rabbit to other rabbit

Nutritional diseases involving calcium and phosphorus deficiency are widespread in rabbit populations. These deficits can cause a variety of complications, including bone diseases, slowed growth, and reproductive problems. Previous research has investigated the effects of age, gender, and biological cycles on the calcium and phosphorus metabolism in rabbits. This review of the literature on the impact of age,

gender, and biological cycles on calcium and phosphate insufficiency in Satin rabbits attempts to compare the results of various research and identify any gaps.

3.3.1.1 Age gender of satin rabbits

Deficiencies in calcium and phosphorus are common causes of nutritional disorders in rabbit populations. These deficiencies may result in several difficulties, such as bone diseases, decreased growth, and issues with reproduction. Prior studies examined how age, gender, and reproductive cycles affected the rabbits' calcium and phosphorus metabolism. In order to compare the findings of numerous studies and spot any gaps, this review of the literature on the effects of age, gender, and reproductive cycles on calcium and phosphate deficiency in Satin rabbits tries to do so. Researchers have also investigated how gender affects the way that rabbits process calcium and phosphorus. Due to the requirements of reproduction, Faria et al. (2016) discovered that female rabbits have a higher demand for calcium and phosphorus than male rabbits. Additionally, it was discovered in this study that compared to male rabbits, female rabbits are more vulnerable to calcium and phosphorus shortages. There was no discernible variation in the levels of calcium and phosphorus between male and female rabbits, according to a(2017)study by Tariq et al.

3.3.1.2 Biological Rhythms

Numerous research has investigated how biological rhythms affect the metabolism of calcium and phosphorus in rabbits. According to Figueiredo et al. (2014), rabbits' calcium and phosphorus metabolism is significantly influenced by circadian rhythms. According to this study, the levels of calcium and phosphorus in rabbits vary during the day, being higher during the day and lower at night. Like this, Melo et al.'s (2020) research discovered that the time of day affects the metabolism of calcium and phosphorus in rabbits, with the morning being the time of day with the highest levels.

3.3.1.3 Comparison

According to the results of these investigations, biological rhythms, age, and gender may all be factored in the development of calcium and phosphorus deficits in rabbits. Due to their increased requirements for certain minerals, young and female rabbits may be more vulnerable to deficiency, and circadian rhythms may also affect their metabolism. The results of these research do not all agree, with some reporting a gender difference that is significant in calcium and phosphorus metabolism and others reporting no gender difference at all. In a similar vein, while some studies revealed a decreasing risk with age, others identified a higher incidence of deficits in young rabbits. In conclusion, the literature on the effects of age, gender, and biological cycles on the metabolism of calcium and phosphorus in rabbits is substantial, however, there are differences in the results of earlier investigations. According to recent research, females and young rabbits may be more vulnerable to calcium and phosphorus deficiency, and circadian rhythms may also affect how they metabolize food. However, more investigation is required to fully understand these results and pinpoint the processes underlying how age, gender, and biological cycles affect the metabolism of calcium and phosphorus in Satin rabbits. This information will enable owners and breeders of rabbits to successfully avoid and treat calcium and phosphorus deficits.

4 Methodology

The study will be conducted on the platform of metabolomic approaches, which will be applied to a series of samples obtained in experimental studies on an appropriately selected group of rabbits with diagnosed dental disease and a group of healthy individuals—the control group. A panel of biomarkers will be created that will serve for the diagnosis and therapy of the mentioned pathological processes, Methodologies on advanced analytical methods HPLC/MS will be developed and, by their application, groups of the above-defined groups of samples will be compared. In addition to the mentioned targeted approach, targeted analysis, an untargeted approach will also be applied to samples. The study will be evaluated using advanced statistical methods, and conclusions will be drawn about the influence of the mentioned biomarkers on the development of dental diseases.

4.1 Selection of suitable animals and sampling

The choice of suitable animals depended on their state of health, coat type and conditions at the clinic. Satin individuals are recognized by their coat, their coat is beautifully shiny. Most animals listed in the category of healthy individuals were collected during preventive castration. However, some in diagnosing other problems that were not related to teeth. Up to two-thirds of the animals were collected under general inhalation anesthesia. Blood was taken QWfrom an artery in the rabbit's ear. The injection site is first disinfected with an alcohol square. After that, an artery is punctured with a needle. Sometimes the pressure in anesthesia is reduced, so you need to be patient and turn the needle a little, sticking to the wall of the vessel. Blood was drawn into a tube with heparin lithium. The approximate amount was 1-2 ml of blood, depending on the situation. Sometimes it was necessary to collect more samples into different types of tubes, not for our purposes, but for further diagnosis of the individual. After collection, it is necessary to fix the ear well with a gauze compress with a self-adhesive bandage, due to subsequent bleeding. When sampling without anesthesia, the assistance of another person was needed, some individuals are not exactly exemplary patients, but due to their pressurization in the blood vessels, the samples went much better. I had to discard a few bloods from my samples because of the sampling procedure. They were taken postmortem directly from the heart, already after the application of the lethal substance, so the blood was assessable. Then I twisted the blood from the test tube in a centrifuge. There was a separation

of blood cells and blood plasma. The sample was described, recorded in electronic form in MS excel, then in a classic paper diary and marked immediately into the freezer.

4.2 Experimental part

(U)HPLC-MS

For the actual development and optimization of derivatization reactions and sample processing procedures, a high-resolution hybrid mass spectrometer LTQ Orbitrap Velos, connected to an Accela 600 quaternary pump and an Accela AS autosampler (Thermo Scientific, USA) was used for the analyses. A TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, USA) connected to an Accela 600 quaternary pump (Thermo Scientific, USA) and an Accela Open AS autosampler (Thermo Scientific, USA) were used for the subsequent measurement of animal samples using the developed methods.

A Gemini C18-NX, 150 x 2.0 mm, 5 µm HPLC column (Phenomenex, USA) was used for the measurements.

Validation parameters (accuracy, correctness, LOD, LOQ, ULOQ) are listed for these substances in Table X.

Table X: Validation parameters for Vitamin D2 and D3 ergocalciferol

Analyte	Accuracy RSD %	Precision RE %	LOD pg/ml	LOQ pg/ml	ULOQ ng/ml
Vitamine D2 ergokalcipherole	16.4	14.6	5	8	600
Vitamine D3 cholekalcipherole	14.6	12.8	4	8	700

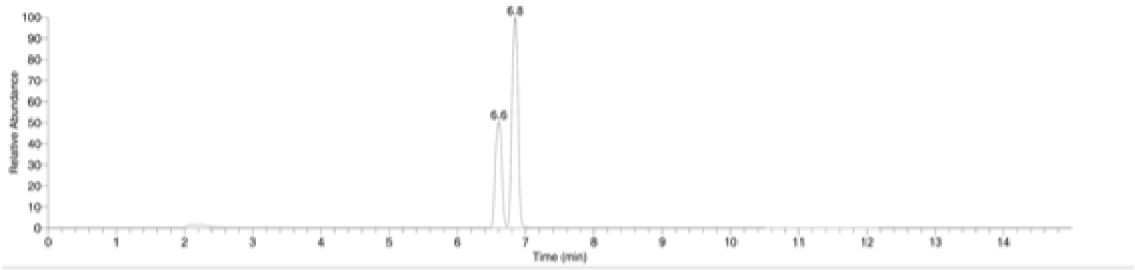

Figure XX: Typical chromatogram of Vitamin D2 ergocalcipherole (Rt = 6.6 min) and Vitamin D3 cholecalcipherole (Rt = 6.6 min)

ICP-OES

The measurements were performed on an ICP-OES 5800 VDV (HP, USA), which allows measurements in both axial and radial directions (VDV mode = vertical dual view). Innovative technology with a vertically positioned burner. Mineralization sample preparation – microwave decomposition of 100 ul of plasma in 10 ml of a mixture of HCl = 1 : 3: HNO3.

MALDI-MS

MALDI-TOFMS experiments were performed on a Voyager DE-Pro 164 (Bruker-Daltonicas, Germany) delayed extraction mass spectrometer equipped with a 337 nm nitrogen laser in positive linear acceleration mode. It was used fresh as a matrix prepared acid 1,2-dimethoxy-4-hydroxycinnamic acid (10 mg/ml) in 50% acetonitrile with 0.1% (v/v) trifluoroacetic acid. The following source parameters were applied: 24 kV accelerating voltage, 76% line voltage and 180 nsec delay. The spectra were taken in the positive ion mode in the mass range m/z 1000 – 35000. An average of 10 x 50 laser shots were counted for one spectrum. The analysis was performed in triplicate. Instrument parameters and laser energy were kept constant during the series of measurements. Experiments were performed on the same day to compare intensity values (cps). Parathyroid hormone and calcitonin - standard were desalted using a ZipTip C18 before the actual MS analysis.

5 Results

5.1 Measured Blood Components in sick and healthy individuals.

In the diagrams below figure 5.1, it shows the values of vitamin D in rabbit blood in individuals affected by dental disease. On the other hand, it is possible to compare with healthy individuals without tooth impairment. In figure 5.2 show the level of calcitonin is high in the healthy rabbits.in figure 5.3,5.4 and 5.5 respectively shows

that calcium, phosphorous and parathyroid is low in the blood of healthy rabbits. Statistically there is a significant difference in the experiment.

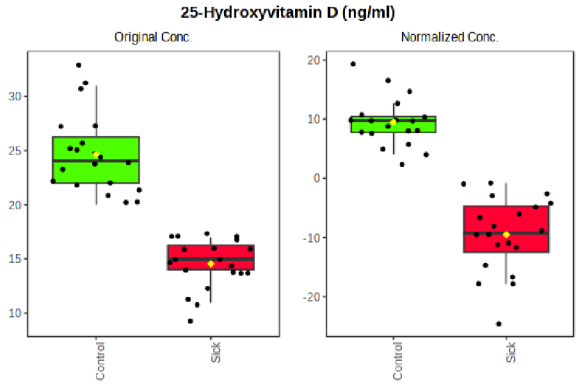

Figure 5.1 Measured 25-Hydroxyvitamin D (ng/ml) in sick and healthy rabbits.

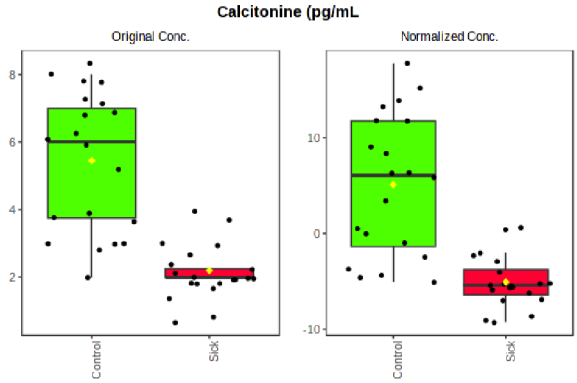

Figure 5.2 Measured Calcitonin (pg/ml) in sick and healthy rabbits.

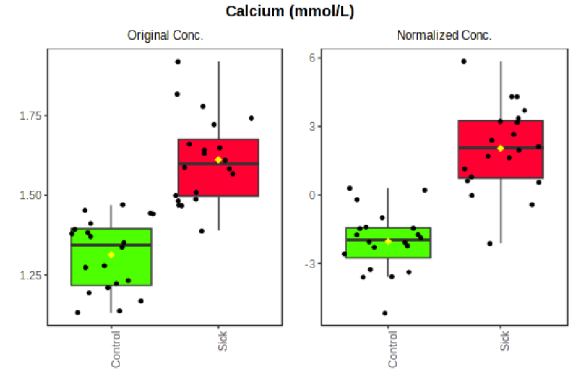

Figure 5.3 Measured Calcium (mmol/L) in sick and healthy Rabbits.

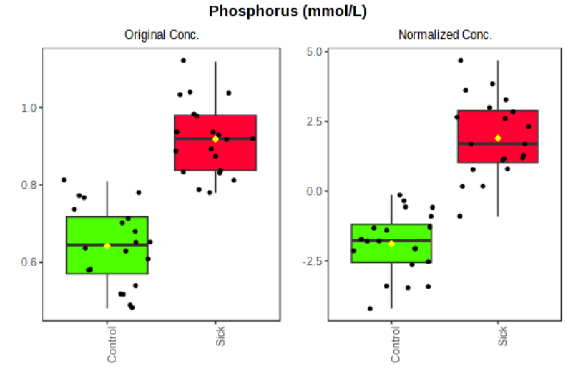

Figure 5.4 Measured Phosphorus (mmol/L) in sick and healthy Rabbits.

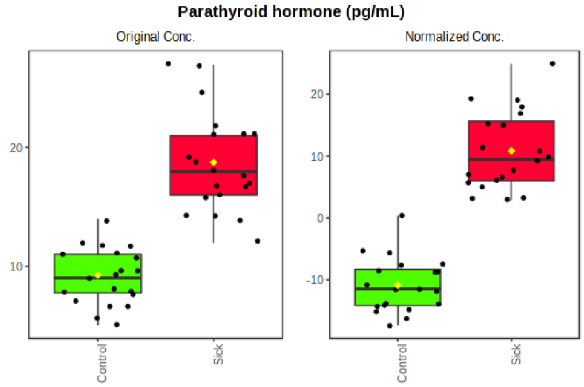

Figure 5.5 Measured Parathyroid hormone (pg/mL) in sick and healthy Rabbits.

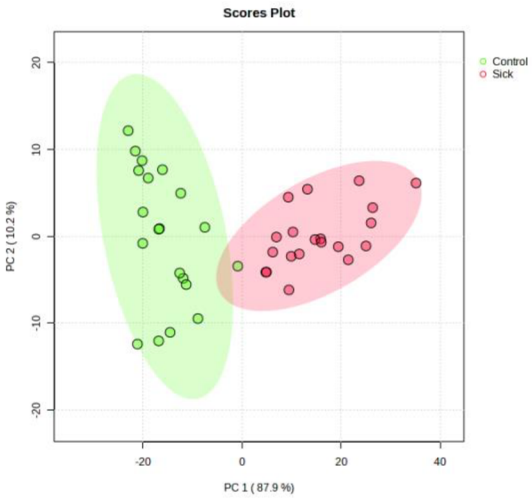

Figure 5.6 scores Plot of PC1 vs PC2 for sick and healthy Rabbits

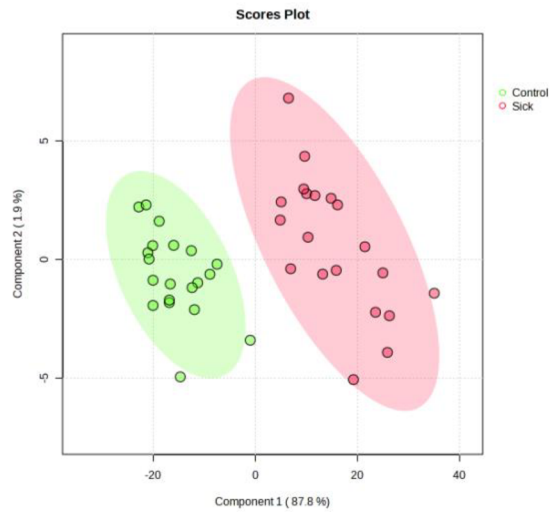

Figure 5.7 Scores Plot of Component 1 vs Component 2 for sick and healthy Rabbits

Name	t.stat	p.value
Parathyroid hormone (pg/mL)	-12.562	4.2183E-15
25-Hydroxyvitamin D (ng/ml)	11.296	1.0395E-13
Phosphorus (mmol/L)	-9.3282	2.274E-11
Calcium (mmol/L)	-7.8814	1.6319E-9
Calcitonine (pg/mL	5.783	1.13E-6

Figure 5.8

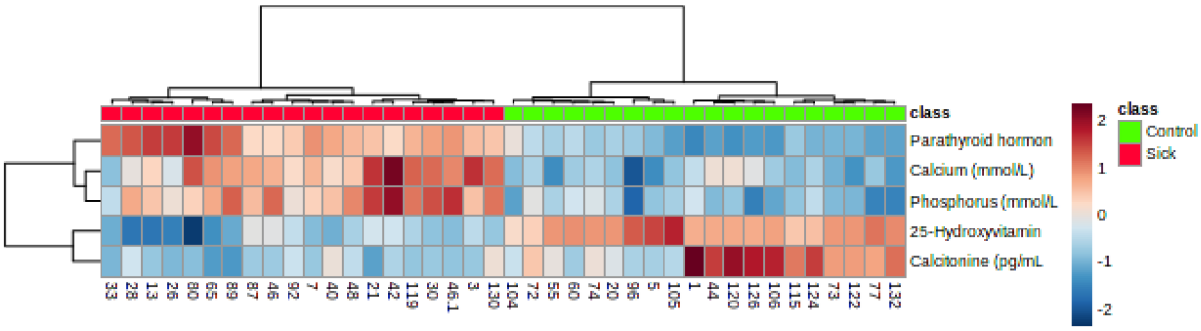

Figure 5.9 Summary of all components for sick and healthy individuals

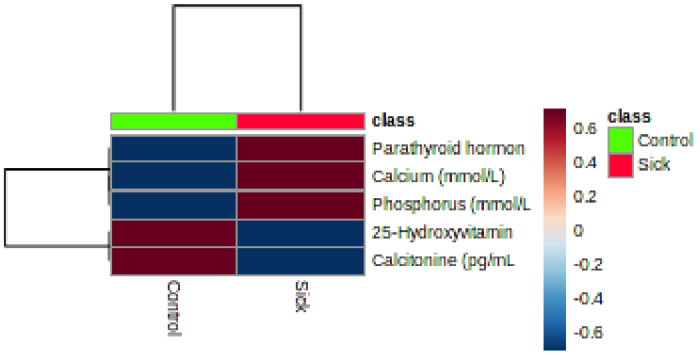

Figure 5.10 Summary of all classes for sick and healthy individuals

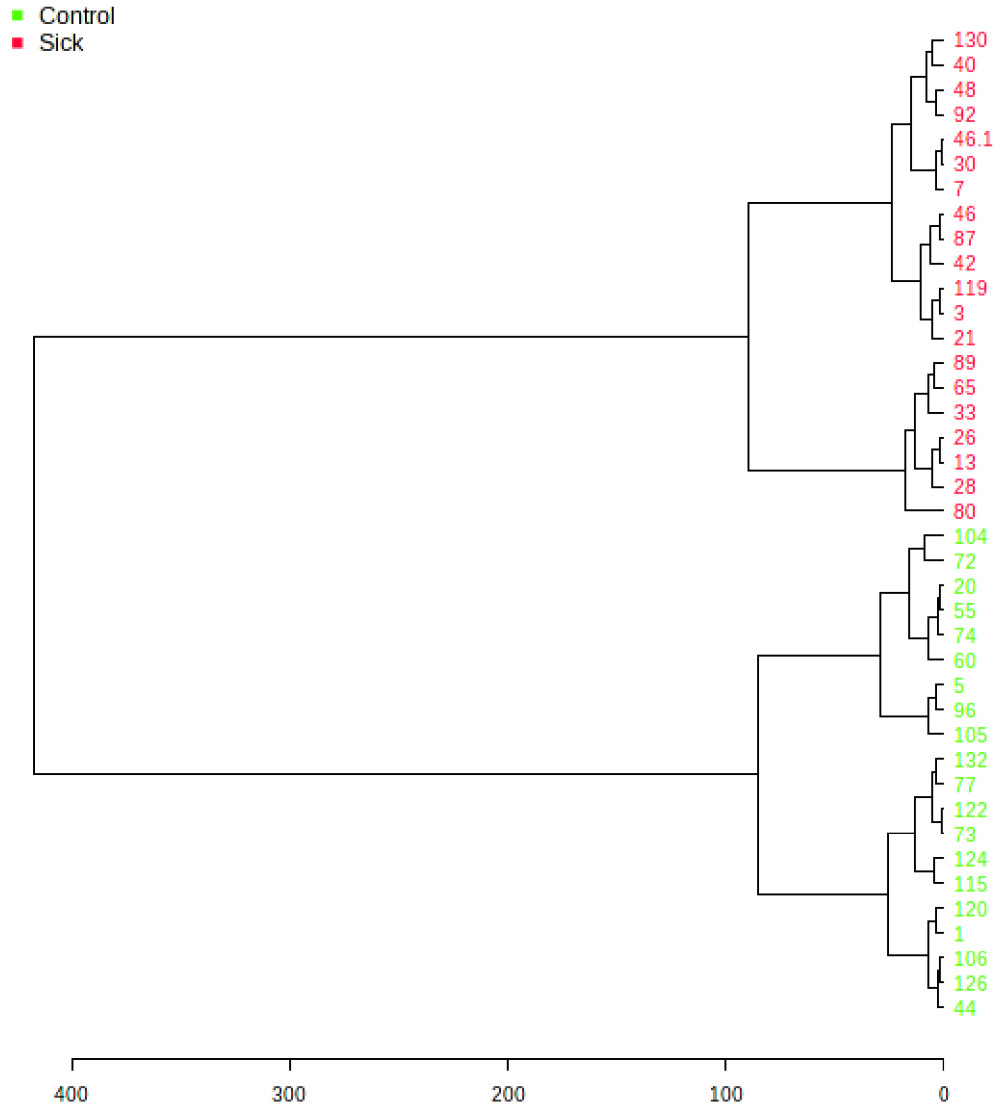

Figure 5.11 Tree showing relationship all components for sick and healthy individuals.

5.2 Measured Blood components of satin and non-satin rabbits.

Figure 5.13 shows that healthy individuals have values slightly higher than animals affected by SOD. The level of calcium, parathyroid, calcitonin and phosphorous in the blood of rabbits with and without satin hair can been seen below with clear difference.

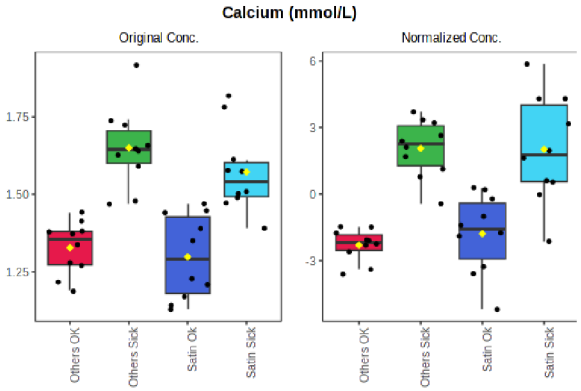

Figure 5.12 Measured 25-Hydroxyvitamin D (ng/ml) in satin and non-satin rabbits

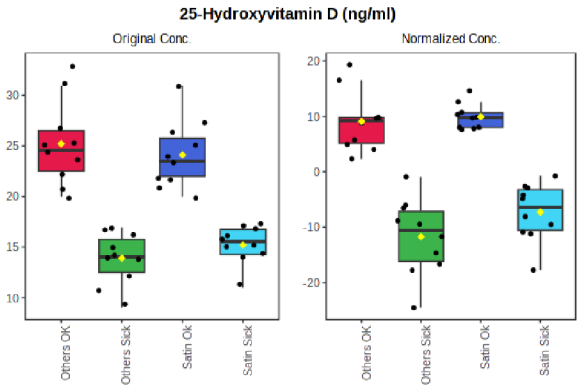

Figure 5.13 Measured Calcium in the blood of satin and non-satin Rabbits

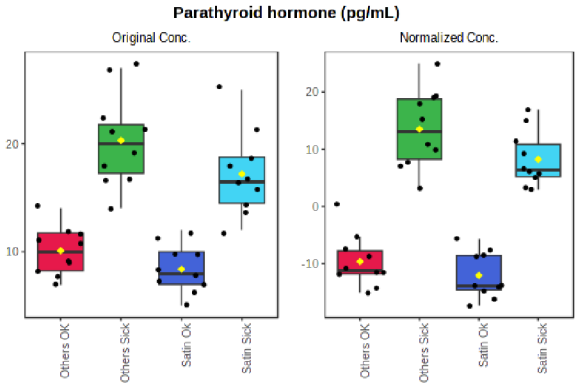

Figure 5.14 Measured Parathyroid hormone in the blood of satin and non-satin Rabbits

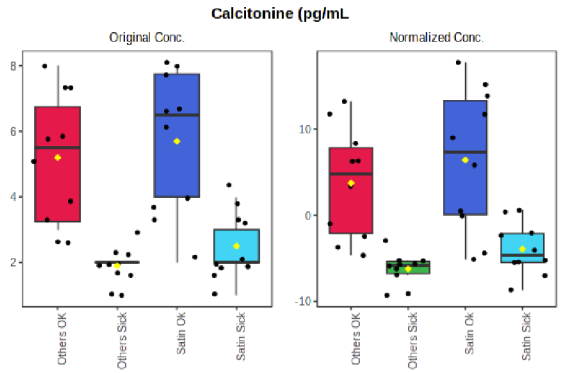

Figure 5.15 Measured Calcitonin in the blood of Satin and non-satin Rabbits

Name	f.value	p.value
Parathyroid hormone (pg/mL)	60.681	3.7301E-14
25-Hydroxyvitamin D (ng/ml)	45.774	2.2667E-12
Phosphorus (mmol/L)	29.05	1.0094E-9
Calcium (mmol/L)	20.04	8.2525E-8
Calcitonine (pg/mL	11.826	1.536E-5

Figure 5.15

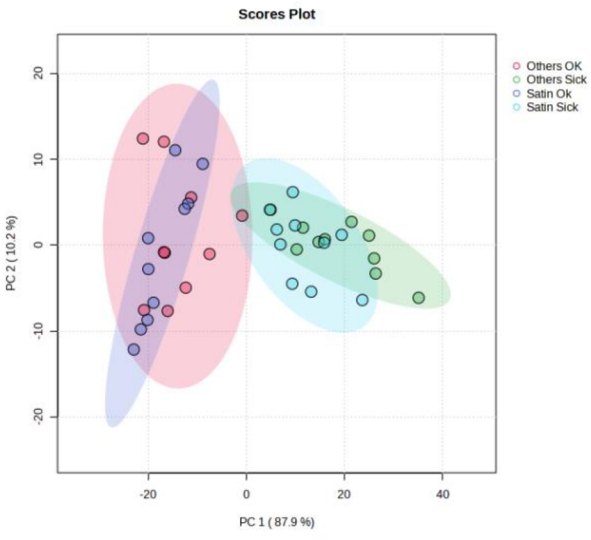

Figure 5.16 scores Plot of PC1 vs PC2 for satin and non-satin Rabbit

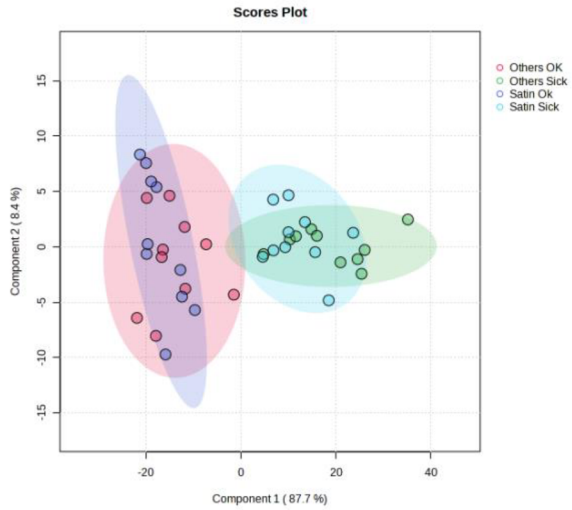

Figure 5.17 Scores Plot of Component1 vs Component2 for satin and non-satin Rabbits.

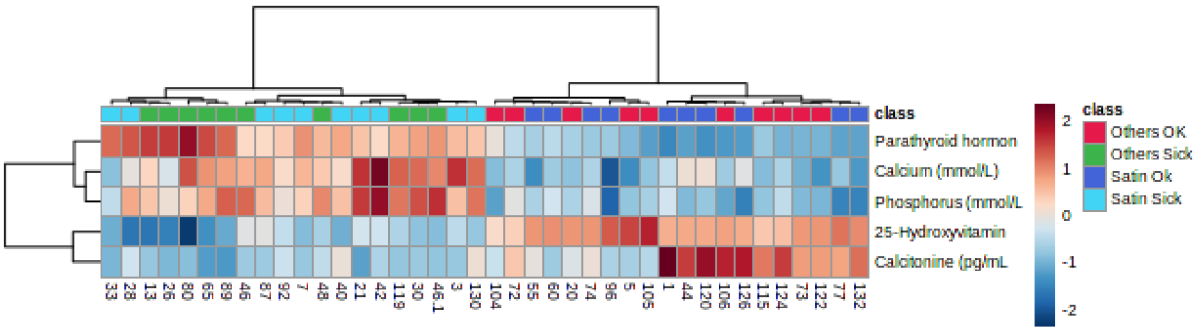

Figure 5.18 Summary graph of all tested components in Satin and non-satin rabbit

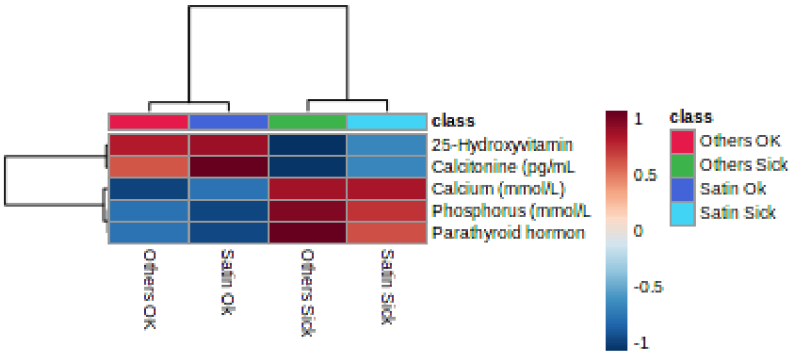

Figure 5.19 Summary class of all tested components in Satin and non-satin rabbit

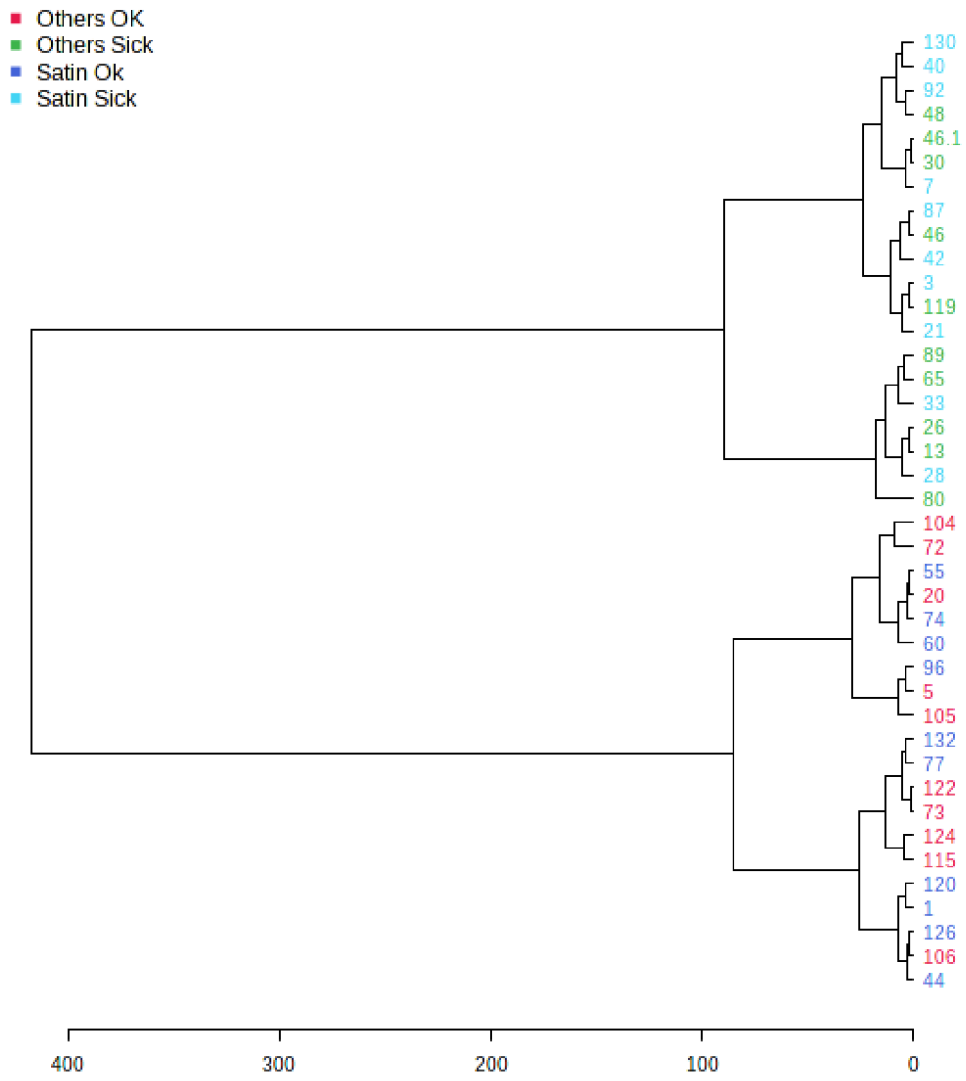

Figure 5.20 Tree showing relationship between all tested components in satin and non-satin Rabbit.

5.3 GENDER ANALYSIS

In all the figures below, there is no significant difference in all the tested parameters.

5.3.1 MALE, MALE NEUTER, FEMALE, FEMALE NEUTER

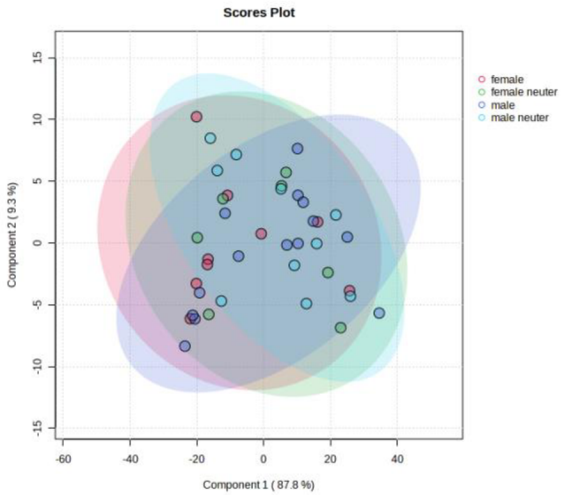

Figure 5.21 scores Plot for all four gender categories

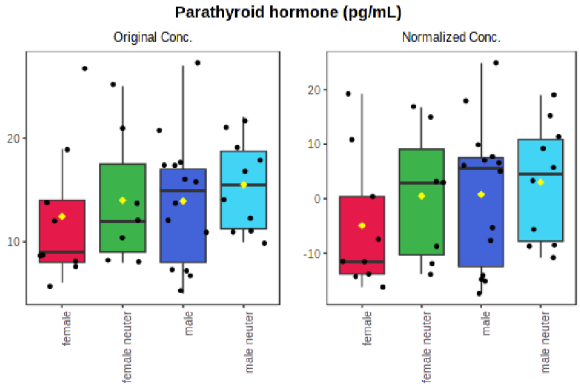

Figure 5.22 Parathyroid hormone in all four gender categories

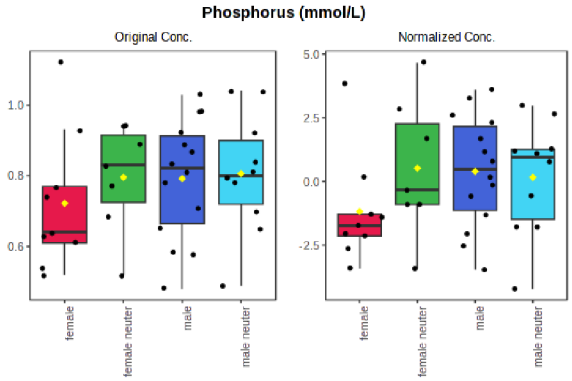

Figure 5.23 Phosphorus measurement in all four gender categories

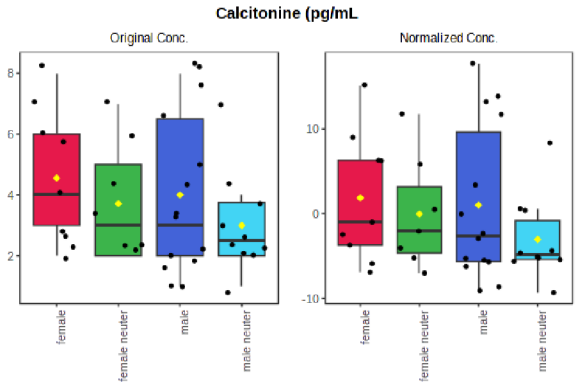

Figure 5.24 Calcitonin measurement in all four gender categories

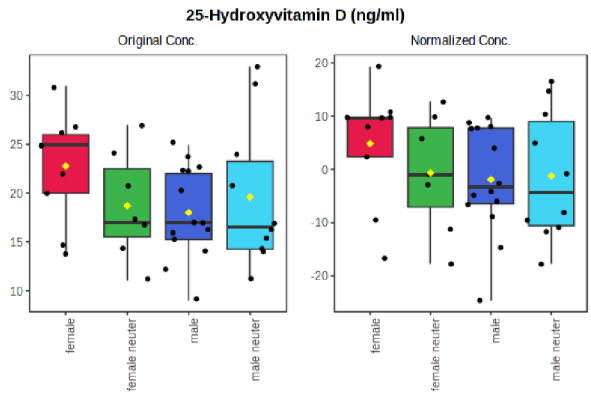

Figure 5.25 Vitamin D measurement in all four gender categories

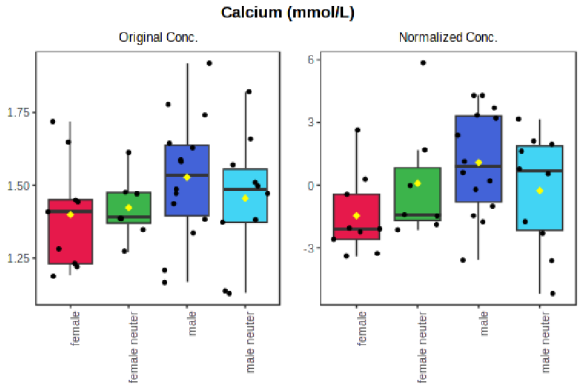

Figure 5.26 Calcium measurement in all four gender categories

5.3.2 MALE, FEMALE ANALYSIS

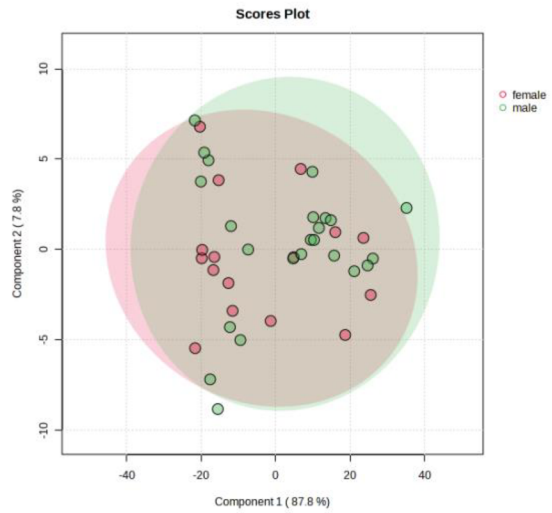

Figure 5.27 scores Plot for Male and Female

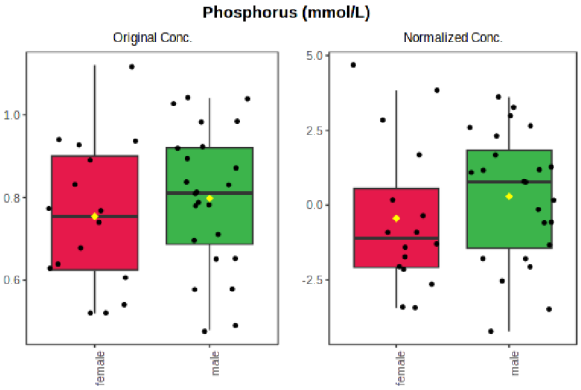

Figure 5.28 Phosphorus measurement for Male and Female gender

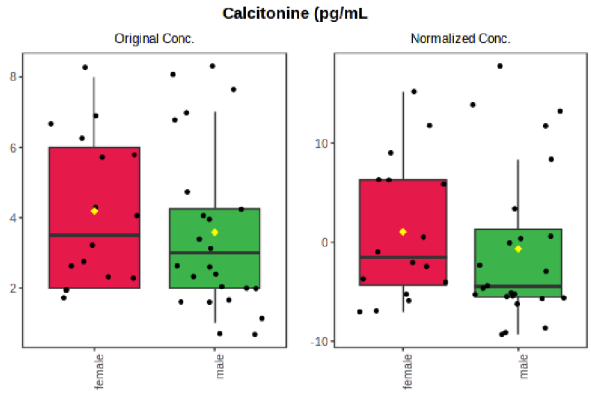

Figure 5.29 Calcitonine measurement for Male and Female

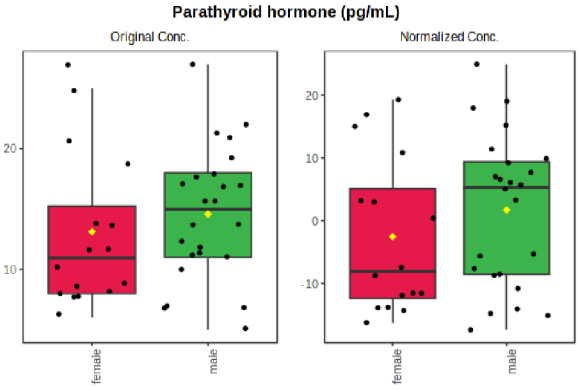

Figure 5.30 Parathyroid measurement for male and Female

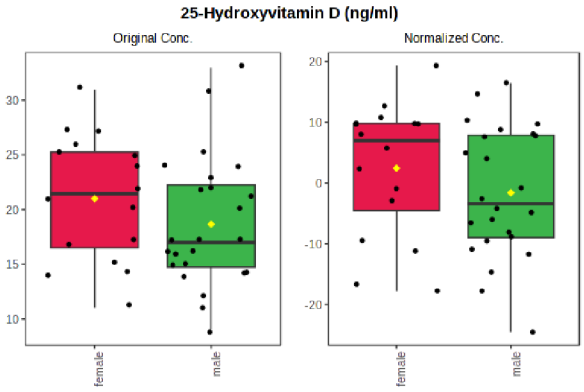

Figure 5.31 Vitamin D measurement for male and female

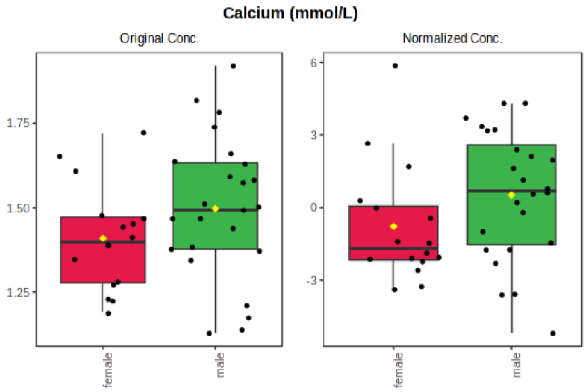

Figure 5.32 Calcium measurement for male and female

5.3.3 MALE, MALE NEUTER ANALYSIS

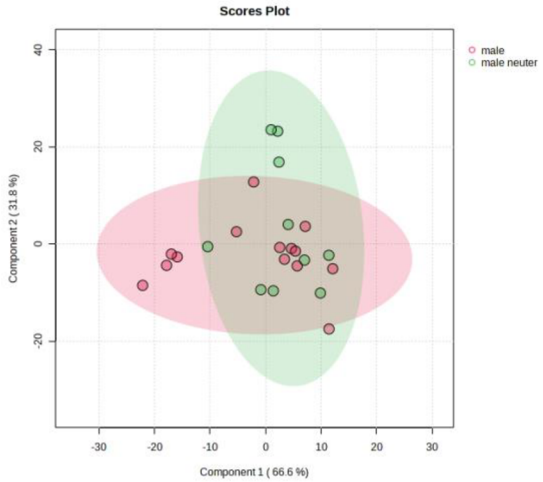

Figure 5.33 Scores Plot for male and male neuter

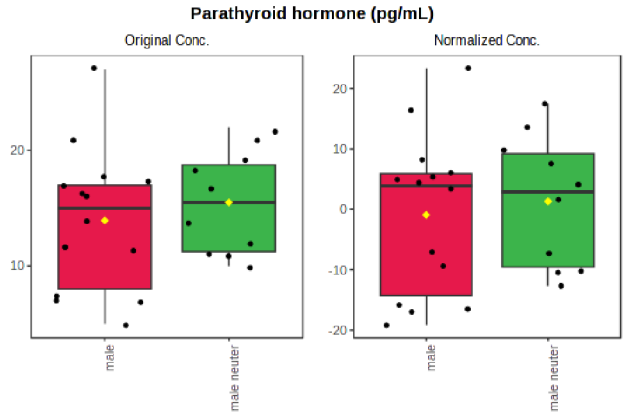

Figure 5.34 Parathyroid hormone measurement in male vs male neuter

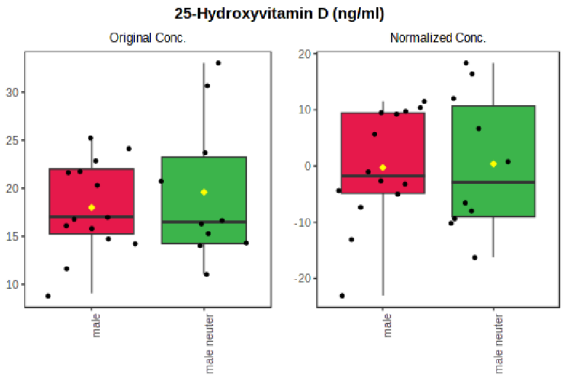

Figure 5.35 Vitamin D measurement in male vs male neuter

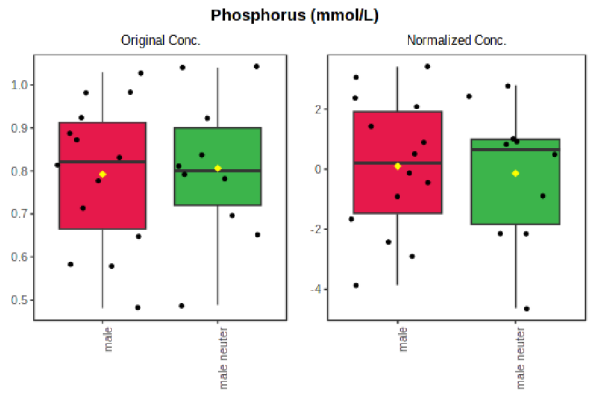

Figure 5.36 Phosphorus measurement in male vs male neuter

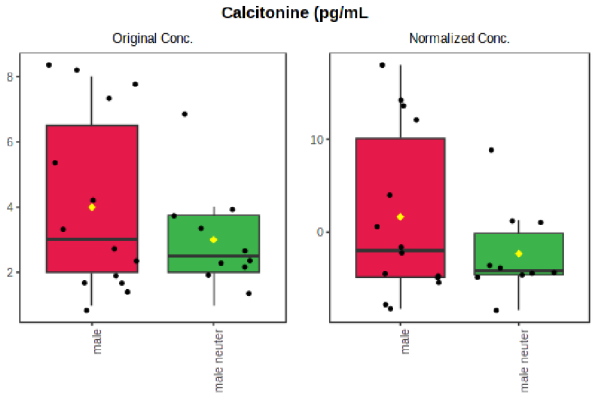

Figure 5.37 Calcitonine measurement in male vs male neuter

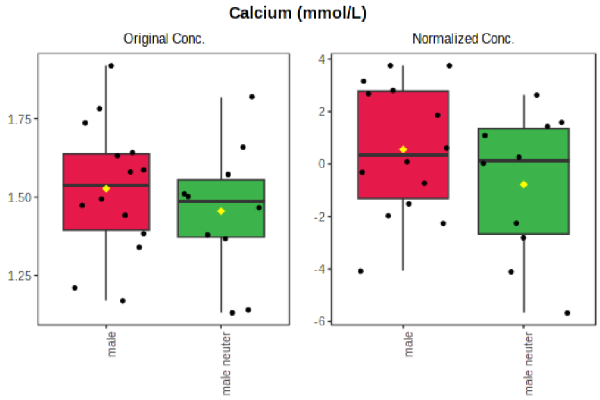

Figure 5.38 Calcium measurement in male vs male neuter

5.3.4 FEMALE, FEMALE NEUTER ANALYSIS

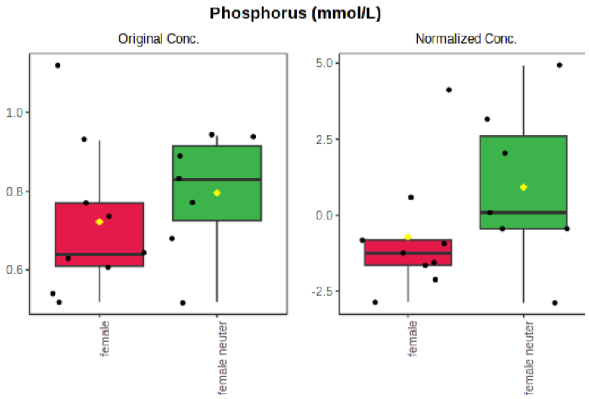

Figure 5.39 Phosphorus measurement in Female vs female neuter

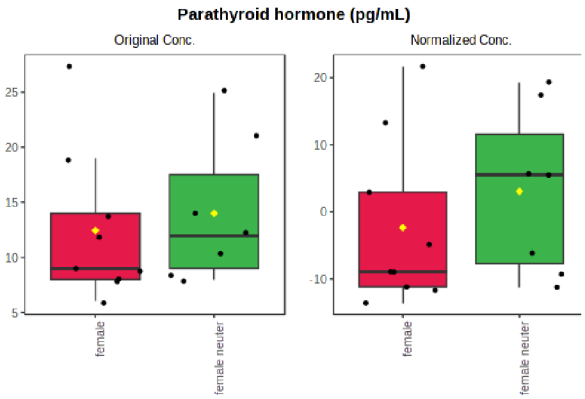

Figure 5.40 Parathyroid hormone measurement in Female vs female neuter

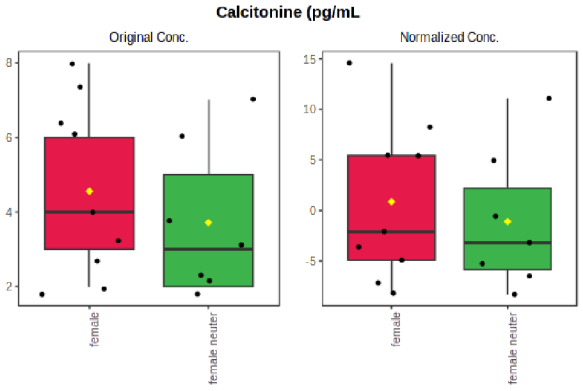

Figure 5.41 Calcitonine measurement in female vs female neuter

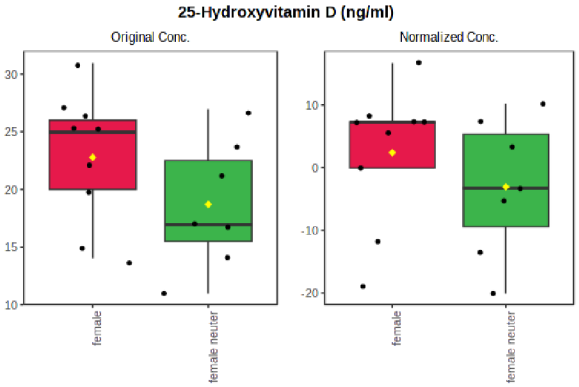

Figure 5.42 Vitamin D measurement in female vs female neuter

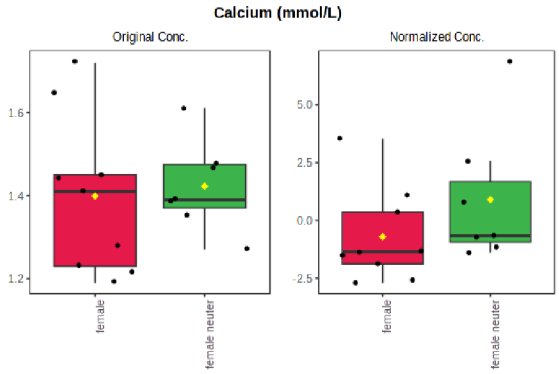

Figure 5.43 Calcium measurement in female vs female neuter

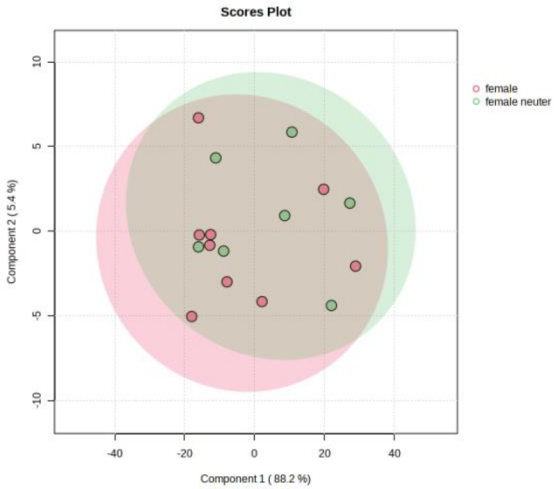

Figure 5.44 Scores Plot of female vs female neuter

5.4 AGE ANALYSIS

In the figures below, the groups are not evenly distributed. There is bias in the statistics.

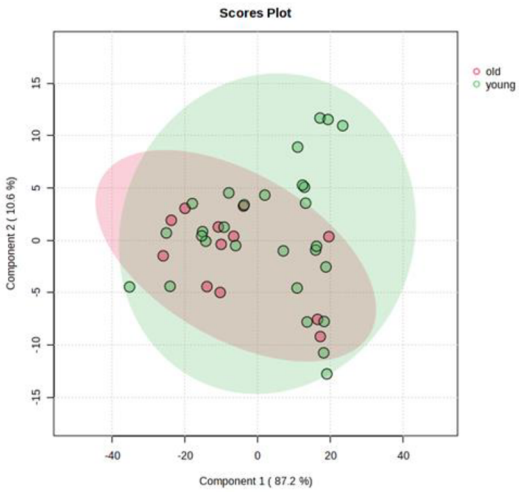

Figure 5.45 scores Plot of old vs Young Rabbit

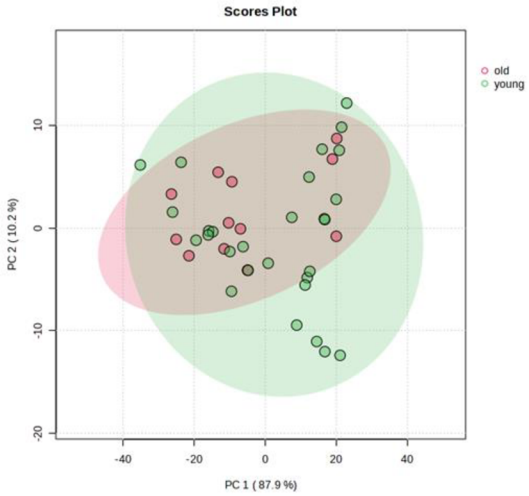

Figure 5.46 scores Plot of old vs Young Rabbit

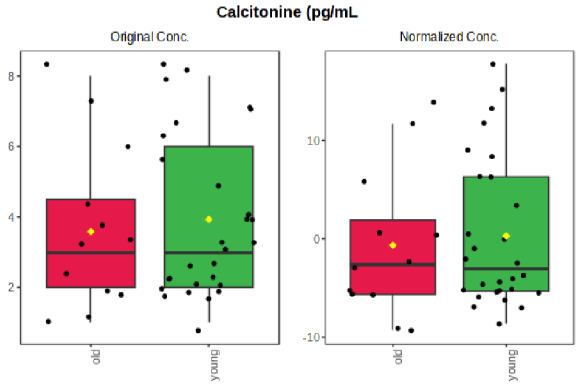

Figure 5.47 Calcitonine measurement in old vs young Rabbit

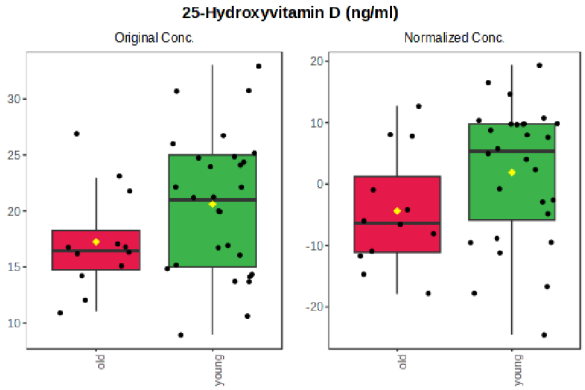

Figure 5.48 Vitamin D measurement in old vs young Rabbits

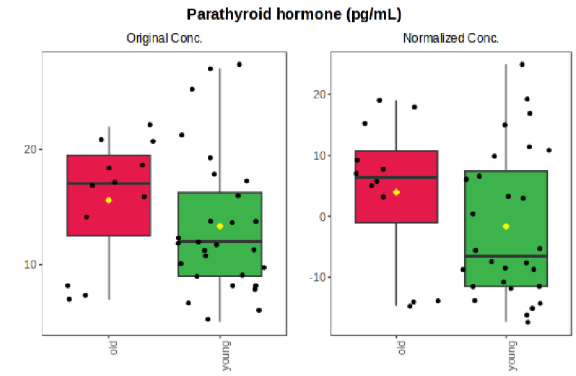

Figure 5.49 Parathyroid hormone measurement in old vs young Rabbits

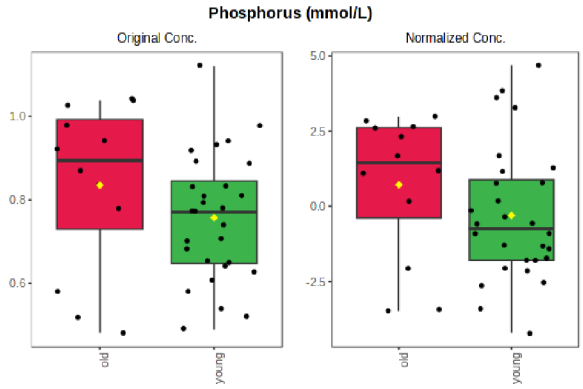

Figure 5.50 Phosphorus measurement in old vs young Rabbits

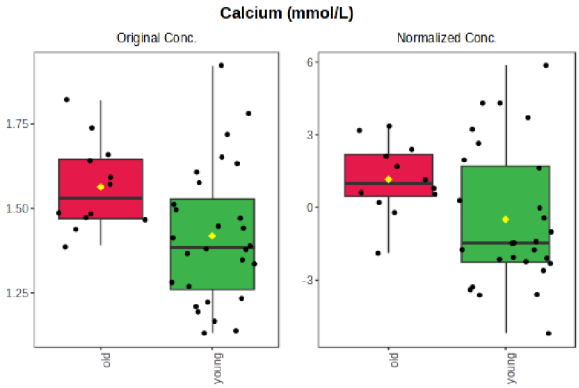

Figure 5.51 Calcium measurement in old vs young Rabbit

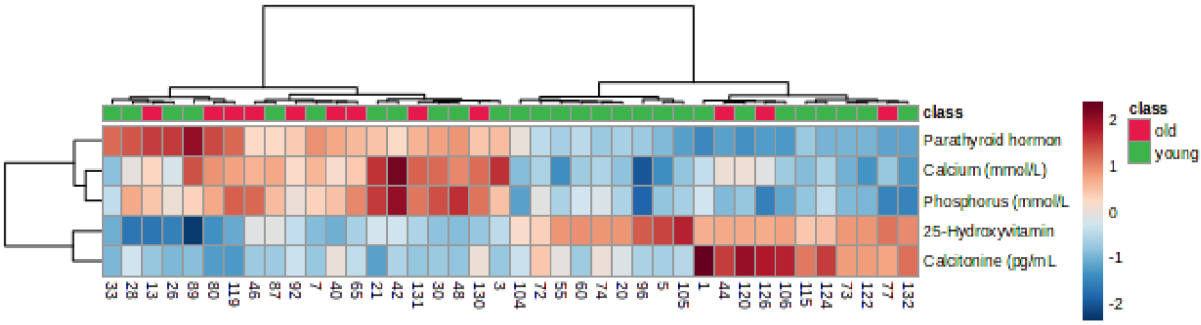

Figure 5.52 Summary of all classes for old and young Rabbits

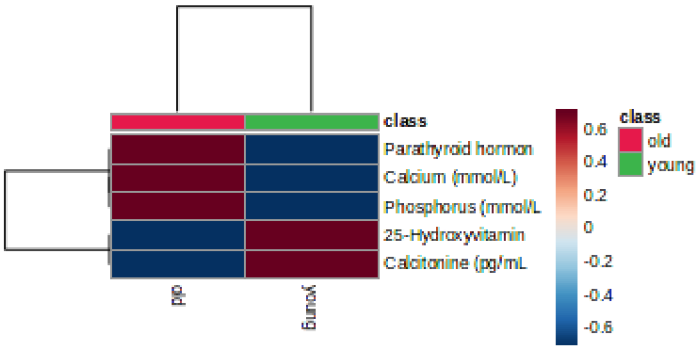

Figure 5.53 Summary of classes for old vs young Rabbits.

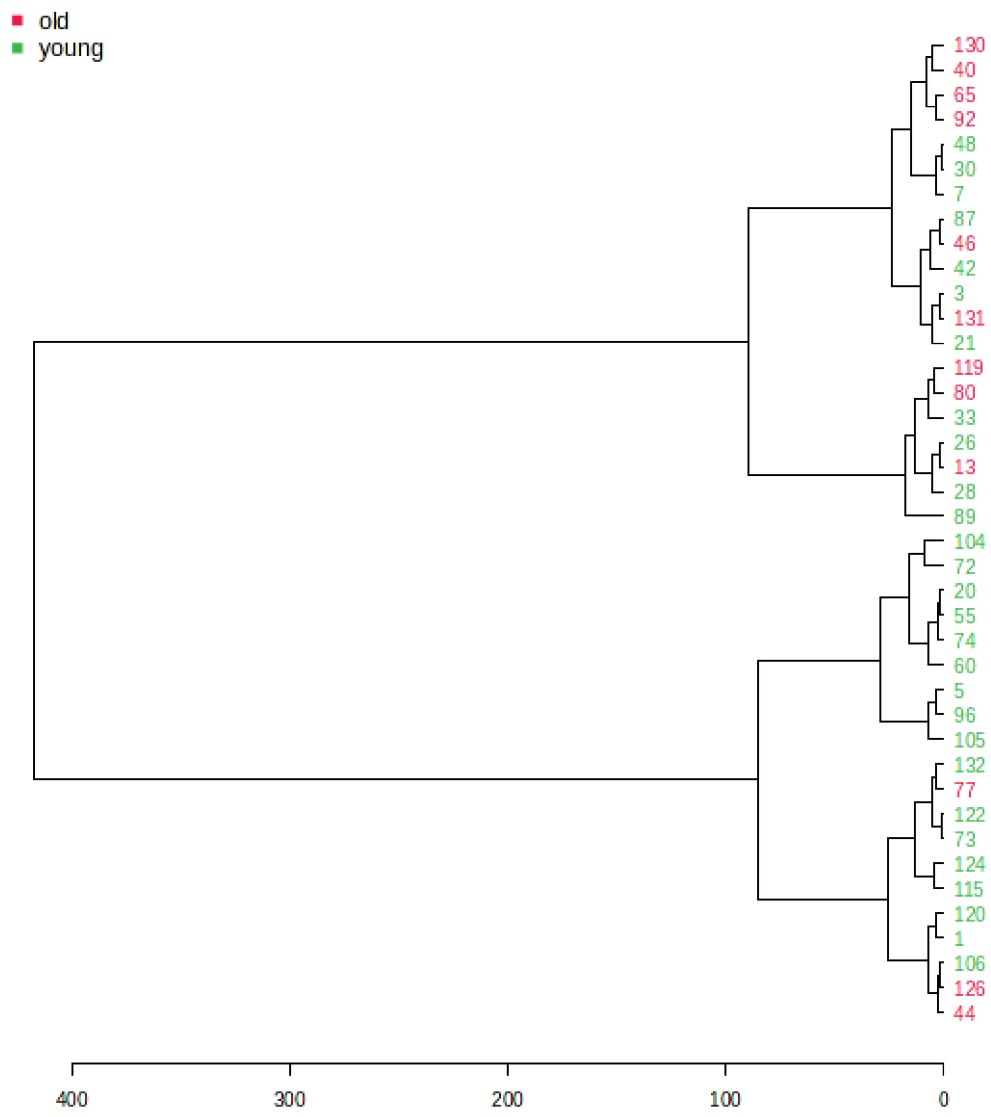

Figure 5.54 Tree showing relationship between the tested blood components in old and young Rabbits.

6 Discussion

In the human body, calcium and phosphorus are essential minerals that play important roles in various physiological processes such as bone formation, muscle contractions, nerve transmission, or cellular signaling. To maintain the balance of calcium and phosphorus in the body, their metabolism is tightly controlled through hormones and vitamin D.

Parathyroid Hormone (PTH): Secreted by the parathyroid glands, increases calcium levels in the blood by promoting calcium release from bones, enhancing calcium reabsorption in the kidneys, and stimulating the production of active vitamin D. Absorption of other nutrients may be impaired by a lack of phosphorus, resulting in more metabolic disturbances.

Calcitonin: Produced by the thyroid gland, lowers blood calcium levels by inhibiting bone breakdown and promoting calcium deposition in bones. Most of the excess calcium is excreted by the kidneys via urine. Calcitonin's primary role is to regulate calcium and phosphorus metabolism, primarily by antagonizing the actions of parathyroid hormone (PTH). Its effect on phosphorus is smaller than that of calcium. Parathyroid hormone (PTH) and calcitonin have opposing effects on calcium and phosphorus metabolism. By inhibiting bone resorption and promoting calcium deposition in bones, calcitonin works to reduce blood calcium levels, while PTH promotes the release of calcium from bones, increases renal calcium reabsorption, and stimulates the production of active vitamin D (calcitriol).

Different factors, such as dietary imbalances, hormonal dysregulation, hereditary causes, and underlying health conditions may cause problems with the metabolism of calcium and phosphorus in rabbits.

Hypocalcemia: refers to abnormally low levels of calcium in the blood. Causes include inadequate dietary calcium intake, vitamin D deficiency, kidney disease impairing calcium reabsorption, and hypoparathyroidism (reduced secretion of parathyroid hormone). Muscle tremors, weakness, seizures, and difficulty breathing can be symptoms.

Hypercalcemia: is characterized by a higher level of calcium in the blood. Excessive intake of dietary calcium, excessive supplementation, and certain tumors, such as lymphoma and hyperparathyroidism, are causes. Symptoms may include lethargy, weakness, loss of appetite, increased thirst and urination, and potentially renal damage.

Hypophosphatemia: is characterized by abnormally low levels of phosphorus in the bloodstream. Impaired dietary phosphorus intake increases renal excretion of phosphorus, and several drugs such as diuretics and malabsorption disorders are the cause. Weakness, lethargy, loss of appetite, and muscle weakness are symptoms.

Hyperphosphatemia: is a condition where the blood phosphorus levels are high. Impaired renal function leads to reduced phosphorus excretion, and certain metabolic disorders are caused by excessive dietary phosphorus intake.

Hyperphosphatemia often occurs secondary to chronic kidney disease in rabbits. Symptoms may include lethargy, weakness, loss of appetite, and potentially calcification of soft tissues (e.g., kidneys).

Calcium-Phosphorus Imbalance: Imbalances between dietary calcium and phosphorus ratios can lead to disorders such as nutritional secondary hyperparathyroidism (NSHP). NSHP is usually due to a diet high in phosphorus and low in calcium, resulting in increased PTH secretion, bone resorption, or soft tissue destruction. The symptoms may include lameness, bone deformities, fractures, or dental abnormalities.

Dental Diseases: Local calcium and phosphorus metabolism in the oral cavity may be disrupted by dental disorders such as tooth decay, malocclusion, or dental abscesses. These conditions may result in changes to bone density, tooth resorption, and alteration of mineral deposits inside the teeth.

In severe cases, a prolonged lack of calcium and phosphorus may be life-threatening to rabbits. To maintain optimum health, it is therefore necessary to provide them with a balanced diet that meets their calcium and phosphorus requirements. The veterinarian is to be consulted for an appropriate diagnosis and treatment if there is suspicion of a deficiency in the rabbit. The treatment of calcium and phosphorus metabolism disorders typically includes addressing the initial causes such as diet changes, supplementation, hormone replacement therapy, and support care. To diagnose and effectively treat these conditions, rabbit owners must work with a veterinarian in close cooperation.

6.1 Sick and healthy rabbits' analysis

The purpose of this study was to investigate the association between various blood factors and animal dental health. We specifically concentrated on five animal metabolism-related variables that are thought to contribute to the emergence of dental issues. Both healthy and ill animals had blood samples drawn, and the data that resulted was evaluated using statistical techniques.

These results imply that blood parameters can be an effective animal oral health indicator. Veterinarians may be able to spot animals at risk of dental issues and treat them appropriately by keeping an eye on these characteristics.

By examining the score pot graphs of calcium, phosphorus, and parathyroid hormone, the current study examined the effect of dental problems on calcium phosphate metabolism in rabbits. The data found that sick people had greater levels of calcium and phosphorus compared to healthy ones. Similarly, parathyroid hormone levels were higher in sick rabbits than in healthy rabbits (Score Pot Graphs, n.d.). These findings suggest that in sick rabbits, calcium phosphate metabolism is impaired, which results in higher calcium levels and subsequent activation of the parathyroid hormone system, which stimulates vitamin D synthesis.

Additionally, Figure 5.1 shows the vitamin D concentrations in the blood of rabbits with dental illnesses. The graph demonstrates the considerable difference in vitamin D levels between sick rabbits (14.5 ng/mL on average) and healthy rabbits (21.4 ng/mL on average). This finding implies that dental disease has a detrimental effect on vitamin D synthesis and may have an impact on rabbit calcium metabolism (Figure 5.1).

These results are in line with earlier studies that have shown how calcium, phosphorus, parathyroid hormone, and vitamin D are interdependent in regulating the calcium phosphate metabolism in animals (Lima et al., 2019). Additionally, research has demonstrated that oral disease can alter the amounts of these hormones and may result in metabolic issues.

Significant findings from the examination of numerous rabbit parameters are shown in Figures 5.2–5.6. Calcitonin parameters are shown in Figure 5.2 to be greater in healthy rabbits and lower in ill ones. Contrarily, calcium levels are high in ill rabbits and low in healthy rabbits in Figure 5.3. In a similar vein, Figure 5.4 demonstrates that phosphorus levels are high in ill rabbits and low in healthy ones. Additionally, as shown in Figure 5.5, parathyroid hormone levels are higher in ill rabbits and lower in healthy rabbits.

The experimental study of vitamin D, calcitonin, calcium, phosphorus, and parathyroid hormone levels clearly indicates a significant difference, as shown by the statistical evaluation of the data in Figures 5.5 and 5.6. Since dental disease in rabbits is known to be connected to calcium metabolism dysregulation, monitoring these parameters in these animals can help control dental disease (Harkness & Wagner, 2010).

The results of these studies demonstrate the significance of keeping an eye on and controlling the levels of calcium, phosphorus, vitamin D, calcitonin, and parathyroid hormone in rabbits, particularly those who are prone to dental disease.

6.2 Satin rabbit and other rabbits' analysis

Interesting results from the examination of satin rabbits are shown in Figures 5.12–5.17. In Figure 5.12, sick satin rabbits have high calcium levels, while healthy satin rabbits have low calcium levels. Additionally, when compared to satin rabbits, normal rabbits without satin had higher calcium levels. In contrast, Figure 5.13 demonstrates that the vitamin D levels in satin and non-satin rabbits are comparable.

According to the examination of parathyroid hormone levels shown in Figure 5.14, non-satin rabbits displayed greater parameters than satin rabbits. Sick satin rabbits had lower concentrations of calcitonin than healthy satin rabbits, while non-satin

rabbits have lower calcitonin levels than non-satin rabbits in Figure 5.15, which tests calcitonin.
The parameters measured in the experiment in satin and non-satin rabbits show clear differences, according to statistical analysis in Figures 5.16 and 5.17. The phenomena have been well-documented in other studies (Faramarzi et al., 2017; Lin et al., 2020), and monitoring these factors in rabbits can aid in preventing dental disorders in satin animals.

These results underline how crucial it is to keep an eye on calcium metabolism in rabbits, especially satin rabbits. In order to prevent dental disorders in satin rabbits, it is essential to investigate the underlying mechanisms that contribute to these variations and to pinpoint prospective treatment options.

6.3 Gender analysis

The third hypothesis of this study suggested that there may be differences in the concentration levels of both elements depending on age, gender, and life biorhythms. The findings of the study, however, showed surprising results, as there was no change in the gender of the rabbit tested. Both neuter male, non-neuter male and neuter female and non-neuter female rabbits showed no clear difference in the statistical examination. The presence of clusters that clearly showed that there was no difference was also observed.
Contrary to earlier research findings, the rabbit model employed in this study did not show gender-based differences in the investigated components' concentration levels. Studies have found gender-based variations in the distribution of trace elements in animals. The findings of this study, however, indicate that gender may not have a substantial impact on these components' concentrations in rabbits. To comprehend the underlying mechanisms behind this discovery, more study may be required.

6.4 Age analysis

The situation in question relates to a statistical analysis of several indicators with respect to gender. The levels of calcium, phosphorus, calcitonin, vitamin D, and parathyroid hormone in males and females were examined.
Surprisingly, the statistical analysis in Figures 5.45 and 5.46 showed there was no discernible divergence in the examined parameters, showing the variables were not statistically different. The fact that this finding deviates from expectations shows that the parameters may not be reliable predictors of differences between young and aged rabbits.

Young and old rabbits' calcitonin levels were carefully examined in Figure 5.47, and it was discovered that there was no discernible difference between the two age groups.

Figure 5.48, on the other hand, showed that young rabbits had higher vitamin D levels than older rabbits did. This implies that the amounts of vitamin D in rabbits may vary with age.
Figure 5.49 examined parathyroid hormone levels and discovered that older rabbits had higher levels whereas younger rabbits had lower levels. This may imply that as rabbits mature, the parathyroid gland's capacity varies.
The amounts of phosphorus were also higher in older rabbits and decreased in younger rabbits, according to Figure 5.50. This can be a sign of alterations in bone metabolism with aging.
Calcitonin levels were especially examined in Figure 5.51, which revealed that older rabbits had higher amounts whereas younger rabbits had lower levels. This may indicate that as people become older, calcitonin function alters.
Finally, a summary graph was shown in Figure 5.52, which demonstrated that the distribution of young and old rabbits in the sample utilized for the analysis was not equal. Only 12 old rabbits and 28 young rabbits were used in the analysis. As a result, it may be difficult to draw firm conclusions about the differences between young and old rabbits. This may have brought bias into the data.

It is significant to note that the sample size and features of the study population may have limitations on the findings obtained from this study. To confirm these results and determine whether they apply to other populations or circumstances, additional research may also be required.

The study also discovered that age-related variations in the evaluated parameters were not randomly distributed, which suggests a bias in statistics. This finding implies that the concentration levels of the investigated components in rabbits may be significantly influenced by age. The results, however, might not be definitive because of the study's uneven distribution of the age groups. Future research should make sure that age groups are evenly distributed to get rid of any potential biases in statistical analysis.
Overall, the findings of this study indicate that gender has no discernible influence on the concentration levels of the investigated components in rabbits, however age may have a considerable impact. To confirm these results and clarify the underlying mechanisms causing these observations, additional study may be required.

7 Conclusion

The goal of the current investigation was to determine whether it was possible to anticipate dental problems in rabbits by tracking the physiological levels of biogenic components and signal molecules that regulate calcium-phosphate metabolism. The study confirmed our first prediction by showing a substantial relationship between the concentrations of these components and signal molecules and the development of dental disorders in rabbits. The prevention and treatment of dental disorders in rabbits is significantly affected by our work. It is feasible to identify and stop dental problems early with targeted therapy or nutrient supplements by tracking the amounts of biogenic components and signal molecules. It is essential to understand the potential toxicity linked to excessive dosage, though. Consequently, thorough nutrition intake control and monitoring should go hand in hand with the use of physiological monitoring.

Our study's findings suggest that physiological monitoring can be a useful technique for the early diagnosis and prevention of dental problems in rabbits. The results contribute to our understanding of the interactions between biogenic components and signal molecules influencing calcium-phosphate metabolism and dental disorders and offer useful insights into the management of rabbit health.

This study shows that, depending on the bio-matrix, there are differences in the amounts of biomarkers related to dental disorders. These biomarkers may be employed as indications for judging the severity of dental problems in rabbits, according to the research. These results underline how crucial it is to keep an eye on calcium metabolism in rabbits, especially satin rabbits. In order to prevent dental disorders in satin rabbits, it is essential to investigate the underlying mechanisms that contribute to these variations and to pinpoint prospective treatment options.

Furthermore, our statistical study revealed no differences between the genders of the rabbits, indicating that gender does not have as much of an impact on rabbit metabolism as was previously thought. However, the results regarding age, might not be definitive because of the study's uneven distribution of the age groups. Future research should make sure that age groups are evenly distributed to get rid of any potential biases in statistical analysis.

The following recommendations are put forth considering the study's conclusions about the effects of gender, age, and biological cycles on calcium and phosphorus metabolism in rabbits:

1. Management of nutrition: Breeders and owners of Satin rabbits should be particularly aware of the nutritional requirements of their animals, particularly during times of rapid growth and development. In order to suit each animal's unique nutritional needs, they should offer a diet that is well-balanced and considers things like age, gender, and biological rhythms.

2. Feeding schedule: Given that biological rhythms have a considerable impact on the metabolism of calcium and phosphorus in Satin rabbits, breeders, and owners should modify their feeding schedules to match these animals' regular feeding cycles. Satin rabbits will get the nutrients they need to maintain good health if the right nutrients are given to them at the right times.
3. Monitoring and assessment: It is essential to regularly monitor and assess the nutritional condition of Satin rabbits in order to quickly identify and address nutritional deficits. Breeders and owners should regularly check the levels of calcium and phosphorus in their animals and change their diets as necessary.

4. Breeders and owners of Satin rabbits can efficiently avoid and manage calcium and phosphorus deficits by adhering to these suggestions, enhancing the health and well-being of their animals.

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9 List of abbreviations and symbols

HPLC High-performance liquid chromatography

MS mass spectrometry