RESPONSE OF GROWING RABBITS IN GROWTH PERFORMANCE AND CARCASS COMPOSITION TO BALANCED DIETARY PROTEIN

By

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Declaration

I, Zenande Mathika, hereby declare that the following thesis, titled "Response of Growing Rabbits in Growth Performance and Carcass Composition to Balanced Dietary Protein", is my original work. The research was conducted at the University of KwaZulu-Natal in South Africa under the supervision of Dr. Z.T Rani and Prof R.M Gous, and it has not been submitted to any academic institution. The contributors to the creation of this work and all sources are fully credited.

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GENERAL ABSTRACT

RESPONSE OF GROWING RABBITS IN GROWTH PERFORMANCE AND CARCASS COMPOSITION TO BALANCED DIETARY PROTEIN

The broad objective of the study was to measure growth performance and carcass composition of growing rabbits as influenced by the dietary protein levels. A 56-day feeding trial was conducted to measure the response of two rabbit breeds in growth performance and carcass composition to balanced dietary protein. A total of 72 sexed rabbits at the weaning age of 5-6 weeks were weighed upon arrival at the farm and randomly distributed singly to 72 grower cages of 61 x 60 x 58 cm. The breeds used were New Zealand White (NZW) and Californian (CAL) rabbits with equal numbers of males and females per breed. A representative sample of 8 New Zealand White rabbits was slaughtered before the beginning of the feeding experiment to estimate the initial carcass composition of the remaining NZW rabbits used in the response experiment. All the remaining rabbits were subjected to the experimental dietary protein treatments that were a result of blending low and high protein basal diets to produce four additional intermediate diets that resulted in a total of six experimental diets (126, 143, 161, 178, 196, 213g/kg). The trial was divided into two periods, from 1-28d and from 29-56d, respectively. Feed intake, body weight gain, and carcass composition were measured. At the end of the feeding trial, 48 rabbits (24 from each breed with equal numbers of males and females within each breed) were sampled for carcass analysis. These were analysed for moisture, ash, lipid, and protein content. Standard methods were used to determine the chemical composition of the rabbit carcasses.

Appropriate regression models, including exponential, quadratic, and linear, were fitted to the data where relevant. The model with the best statistical fit was selected. Daily feed intake and final body weights were significantly influenced by the dietary protein levels ($P < 0.05$). Dietary protein did not influence feed conversion efficiency (FCE), ($P > 0.05$). The highest feed intakes, body weight gain, and consequently FCE's were observed in the NZW breed. The NZW male rabbits exhibited highest feed intake and body weight gain while NZW female rabbits had the highest FCE. Significant interactions were detected in feed intake and body weight of the two breeds ($P < 0.05$). Female rabbits of the two breeds showed a significant interaction in feed intake and final body weight to the dietary protein levels.

Moisture, ash, lipid, and protein in the carcass were not affected by the dietary protein content ($P > 0.05$). No significant (breed x sex) or (protein x breed x sex) interactions were observed in the carcass composition parameters. CAL rabbits had higher ash, lipid and protein and lower moisture contents than the NZW rabbits. Fat content was increased as the dietary protein content was reduced ($P > 0.05$). As a result, both males and females of the NZW breed had highest lipid contents on the lowest dietary protein level. CAL females and males had higher protein contents than NZW female and male rabbits, respectively. Carcass and pelt weights exhibited a significant response to the dietary protein levels ($P < 0.05$). Significant interactions in carcass and pelt weights were observed ($P < 0.05$).

Considerable variation within treatments in all responses measured in this study meant that the responses to dietary protein could not be accurately described. Solutions to this problem would be to use more rabbits per treatment, to sample more rabbits per treatment, and to check on the accuracy of the laboratory analyses. Widening the range of dietary protein levels may result in a greater difference in the response of the rabbits to protein, thereby describing this response more accurately.

Keywords: Rabbits, dietary protein, feed intake, chemical composition, breed.

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Dedication

I dedicate this thesis to my late grandmother (Nojongile Xoliswa Mathika), who raised me to be woman that I am today who believes in herself no matter what. I also dedicate this work to my supportive family, Anti (Nomanene Rheme), Uncle (Andile Mathika), aunt (Sivenathi Rheme) and my brother (Ntlahla Mathika).

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List of abbreviations

AAs	Amino acids
ADFI	Average daily feed intake
ADG	Average daily gain
AOAC	Association of Official Agricultural Chemist
ARC	Agricultural Research Council
BDP	Balanced dietary protein
BWG	Body weight gain
CAL	California
CP	Crude protein
DLys	Digestible lysine
EAAs	Essential Amino Acids
FCE	Feed conversion efficiency
G	Grams
g/kg	Gram per kilogram
HP	High protein
Kg	Kilograms
LP	Low protein
Lys	Lysine
Mj	Mega joules
N	Nitrogen
NEAAs	Non-Essential Amino Acids
NZW	New Zealand White
Pm	Protein mature weight
SEM	Standard error of mean

Chapter 1

General Introduction

1.1 Background

A project to model the feed intake and growth of rabbits has been launched at the University of KwaZulu-Natal. Simulation models are a potential strategy for nutritionists to increase production performance and profitability by integrating genetic, environmental, and nutritional parameters simultaneously to evaluate nutrient needs and estimate feed intake and performance under various scenarios. Miller and Payne (1963) provided evidence of the effectiveness of the use of models in interpreting and predicting animal responses to dietary inputs. The model being developed is based on the food intake theory put forward by Emmans (1981; 1987), which assumes that an animal will try to grow along its potential path to reach sexual maturity as soon as possible. The theory put forth by Emmans (1981) to estimate voluntary food intake in pigs and chickens is unquestionably the most significant subsequent contribution to response modelling because it greatly increased the relevance of prediction models by making food intake an output from the growth model rather than an input. The animal will attempt to consume sufficient of a given feed to enable it to reach this aim, but it may be prevented from doing so if it reaches its gut capacity or if it fails to adequately release heat to the environment. In broilers and growing pigs, this approach has been successful in predicting food intake and growth (Ferguson *et al*.,1997). The creation of mathematical models that describe the growth of broilers to determine responses to production adjustments techniques and maximization of profitability have received consideration attention (Berhe and Gous, 2008).

In order to develop such a model successfully for growing rabbits, it is necessary to describe the growth rate potential of an animal and the limits to changes in body composition, particularly lipid, in response to changes in feed composition. When an animal is presented with a feed limiting in protein it will over-consume energy to consume sufficient of the limiting nutrient (Emmans, 1987) but there is a limit to the amount of body lipid animals can deposit, this amount differing between and within sexes and species (Gous and Brand, 2008). By subjecting growing rabbits to a range of dietary protein contents and measuring their feed intake and growth of body components, these limits to body lipid deposition may be measured. Hence, the need to measure the response of growing rabbits in growth performance and carcass composition to balanced dietary protein.

1.2 Problem statement

The primary objective of this research study is to address the significant knowledge gaps concerning the use of simulation modelling to estimating feed intake, daily weight gain, and feed conversion efficiency in growing rabbits. Specifically, the study seeks to evaluate how growing rabbits of two breeds respond to balanced dietary protein levels. While simulation modelling has been successfully used in estimating growth performance and carcass composition in poultry and pigs, there is a dearth of research in this area for rabbits. Thus, this study aims to provide useful data that can be incorporated into simulation models for growing rabbits. Furthermore, the research also aims to investigate the optimum levels of dietary protein that can be included in growing rabbit diets to enhance growth performance and carcass composition. This aspect of the study is critical since protein is a vital nutrient in animal diets and plays a significant role in determining the growth performance and carcass composition of growing animals. Thus, the research findings could provide essential insights into the formulation of optimal rabbit diets that can improve animal productivity and profitability. Overall, the study is designed to address important gaps in the current knowledge of simulation modelling for growing rabbits and determine the most suitable dietary protein levels for optimal growth performance and carcass composition. The results of this research will be beneficial to animal nutritionists, rabbit producers, and the animal feed industry at large, as they can use this information to develop more efficient feeding strategies for growing rabbits.

1.3 Justification

There is little to no literature available on the response of growing rabbits to balanced dietary protein. However, prediction of feed intake, growth rate and body composition is a common challenge in animal production. Simulation models that predict performance are the answer to this problem. Science has evolved for so many years and has resulted in the creation of models that explain the performance of livestock. Models such as the Edinburgh Model Pig have been used to predict pig performance and this has paved the way for further model development. It is now advantageous to adopt the use of growth models which have become more common in the poultry industries and this in turn helps nutritionists and producers to make predictions of animal performance when growing animals are given a particular feed or exposed to a certain feeding regime. Apart from the potential to maximize farm profitability and production through the use of simulation models, there are other significant benefits to be obtained from the process of constructing a simulation model, particularly when it is integrated into the research process.

Additionally, models can be helpful in identifying areas for future research and in avoiding the waste of time and resources on further experiments that will only generate information that is already known. The theory used to simulate food intake (Emmans, 1981) has had significant benefits for modelers because it has been successfully applied in simulating the influence of, among others, changes in dietary amino acid and protein content, environmental temperature, infection, and social stress. This raises the chance of the rabbit model being researched and developed to add to the scientific knowledge about rabbit production opportunities. This project is part of a larger project aimed at producing a mechanistic model of the food intake and growth performance of rabbits. Because such information is essential in the development of a simulation model of food intake and growth of rabbits, a protein response trial needs to be conducted.

1.4 Objectives

The broad objective of the study was to measure the response of growing rabbits in growth performance and changes in the growth of the physical and chemical components (moisture, ash, lipid and protein) as influenced by dietary protein content.

The specific objectives were to:

1. Measure the response in growth performance (feed intake, body weight gain, and feed conversion efficiency) as influenced by the dietary protein levels.

2. Measure the response in carcass composition (moisture, ash, lipid and protein) and changes in the growth of the physical (carcass and pelt weights) as influenced by dietary protein content.

1.5 Hypothesis

The hypothesis tested in this study is that:

1. Reduced dietary protein levels will result in increased feed intake, body weight gain and feed conversion efficiency.

2. Lowest dietary protein level results in improved carcass composition (lipid, protein, ash, and moisture content).

Chapter 2

Literature review

THE IMPORTANCE OF DIETARY PROTEIN FOR GROWING RABBITS

2.1 Introduction

Growing animals require resources from the feed offered and to be kept in a favourable environment if they are to reach their full growth potential. The key premise is that, under non-limiting conditions, a growing animal will seek to consume enough of the feed provided to reach this potential (Emmans, 1981). According to Emmans (1987), growing animals require dietary protein (amino acids) for maintenance and growth, and energy for maintenance, growth, and lipid gain, assuming that other nutrients are available in sufficient amounts.

The composition of the body of an animal is generally assumed to change both physically and chemically during its growth. However, except for the observation that variations in mature size do occur among animals (Taylor, 1980) and that selection might affect mature size, growth rate, and weight gain at a given level of maturity, there has in the past been no common agreement or discussion in the literature on approaches of identifying genotypes that would enable animals to be compared (Emmans, 1989). This has changed in the past two decades, and it is becoming common practice to make use of the Gompertz equation to describe potential growth. This equation has been shown to be the most suitable equation for describing the potential growth of the body and its components (Emmans, 2022). As a result, experiments have been performed by various authors to describe the Gompertz characteristics of various genotypes available to the broiler sector when grown under non-limiting conditions (Hancock *et al*., 1995; Gous *et al*., 1999; Vargas *et al*. 2020), as well as those available to the pig industry by Ferguson and Kyriazis (2003).

Once the potential growth of a genotype is known it is possible to describe the potential rate of protein growth in the body and that of the various physical components of the body. The chemical components other than protein, and all the physical components, are allometrically related to body protein if they share the same rate of maturing (B in the Gompertz equation) (Emmans, 1988). These relationships are part of the description of potential growth for a given genotype.

In poultry, all the physical parts of the bird mature at the same rate as body protein except for feathers (Emmans and Fisher, 1986). There is no information in the literature regarding the

rate of growth of the rabbit pelt in relation to the growth of body protein, and this must be investigated before pelt growth can be predicted. The amino acid composition of the rabbit pelt differs from that of the body of the rabbit, so separate equations would be required to calculate the amino acid requirements of these two components.

The protein requirement for body maintenance is suggested to have an identical composition to that of the body (Emmans, 1989). He suggested that the maintenance protein requirement (*MP* g/d) is related to a scaled maintenance unit similar to that used for determining the energy required for maintenance, calculated as $MP = M_p P_m^{0.73} u$, where P_m is the mature body protein weight, u is the degree of maturity, P_t/P_m. The value of M_P is assumed to be 8 g per unit day. In poultry, the protein requirement for feather maintenance is assumed to be a rate of 0.01g/g feathers a day. The maintenance requirement for the maintenance of rabbit pelt could be calculated in a similar way, but no attempt has been made to do so in the literature.

In an experiment conducted by Hancock *et al*. (1995) to measure the potential growth rates of females and males of 6 broiler commercial strains, it was discovered that commercial broiler genotypes have different potential growth rates, which implies that these genotypes have varying nutritional and environmental needs that must be considered if attempts are made to grow these various genotypes to their potential.

Dietary protein concentration greatly influences feed intake, consequently growth performance and carcass characteristics in broiler chickens (Liu *et al.,* 2017). Growth rate and carcass composition will vary as the dietary protein is increased due to different protein levels supplied and the amount of feed being consumed at each level. Rabbits are likely to show different responses to dietary protein levels. Because dietary protein content influences the cost of the diet, growth rate, feed intake and carcass composition, various authors have demonstrated that the protein content of the diet has a considerable impact on feeding costs and revenue hence profit in a broiler enterprise (Skinner *et al*., 1991; Smith *et al*., 1998; Eits *et al*., 2005).

The cost of each protein-containing feed ingredient will vary from time to time. Feed intake, growth rate, and carcass composition will also differ between rabbit breeds and as dietary protein content varies. Due of these variations, the revenue made from selling the rabbits will also differ, as well as the profit (income minus feeding cost). Feeding costs are calculated as feed intake x cost/kg of that feed. Revenues depend on how the animals are marketed, as live, eviscerated carcasses or cut-up parts. Despite the fact that rabbit carcasses are also sold whole, retail cuts continue to gain importance. In addition, as cited by Hoffman (2004), Sonandi *et al*.

(1996) also stated that most consumers prefer rabbit meat to be sold as cut up parts as they imply that whole rabbit carcasses resemble an image of an infant or that of a cat

Figure 2.1 An image of a whole rabbit carcass, Source: (Candelaria-Martinez *et al*., 2021)

It is likely, as the dietary protein content in rabbit feeds is increased, that the response in profit will reach a peak and then decline, making it possible to determine the optimum economic level of protein to feed to rabbits, i.e., where the income minus feeding cost (sometimes called margin over feed cost) is maximised. This is what a rabbit producer would like to achieve, but it will vary, depending on the cost of dietary protein and the revenue obtained from selling the rabbits; when the price of protein increases, the margin will be reduced, and the optimum protein level may change. This is likely to occur if the cost of rabbit meat increases or decreases, the margin might change as a result. In order to calculate the margin over feed cost it is essential to have an idea of how the growing rabbit responds in food intake, growth and carcass composition to dietary protein. Literature on the response of growing rabbits to balanced dietary protein is either scarce or non-existent, hence much of the information from previous studies on responses of growing broilers and pigs to dietary protein has been used in this review. For these reasons, it is important to measure the response of growing rabbits to balanced dietary protein.

2.2 Amino acid requirements for maintenance and growth

Understanding the amino acid and protein requirements of domestic animals is a fundamental aspect in formulating cost-effective and efficient diets, as highlighted by Carabaño Luengo *et al*. (2009). The nutritional needs of animals depend on various factors such as age, genetics, and environment, and therefore, it is crucial to determine their dietary requirements at different stages of their growth and development for optimal production outcomes.

To achieve maximum efficiency and reduce nitrogen excretion, nutritionists and modellers have been striving to predict the response of birds to nutrients. Research has mainly focused

on determining the nutrient requirements of groups of birds and developing nutritional strategies that align with their needs. The concept of a 'nutrient requirement' has been extensively used in poultry nutrition to determine the ideal nutrient content required to achieve the highest level of production. However, according to Fisher (2008), the concept of a 'nutrient requirement' is no longer adequate, and the approach needs to change to align with the objectives of the company. Instead of focusing solely on maximizing production levels, nutritional decisions should consider the overall goals and objectives of the company. Therefore, it is necessary to shift from the outdated approach of a 'nutrient requirement' to a more holistic approach that considers the overall goals of the company.

The estimation of amino acid requirements of broilers has received a significant amount of attention and most of this research makes use of a graded supplementation method to evaluate responses to a limiting amino acid (Gous, 1990). D'Mello and Lewis (1970) provided evidence showing that the requirement of one amino acid may be dependent on the amount of another amino acid proposing that all amino acid requirements need to be considered together. According to van Milgen and Dourmad (2015), factorial approach and experimental empirical methods are the two main methods for determining amino acid requirements. The factorial approach uses the AA composition of retained protein to estimate the necessary amount of amino acids (AAs), which is considered to remain constant. Nevertheless, Fisher *et al.* (1973) have demonstrated that the animal AA needs are changing rather than constant. The requirement of protein for both maintenance and growth are the total amino acid needs. According to Emmans and Fisher (1986), the only pragmatic classification of animal features for determining nutrient requirements is potential performance as influenced by genotype and state of a bird.

Maintenance is defined as the amount of a specific nutrient required to maintain the current condition of an animal, with no gain or loss. Amino acid requirements for maintenance are often determined at nitrogen (N) equilibrium, which occurs when N intake precisely equals the total of N losses, resulting in a constant N content in the body (Sakomura and Coon, 2003). Black *et al.* (1986) concluded from previous studies that the maintenance requirements are influenced by sex and breed.

Plavnik and Hurwitz (1983) discovered that amino acid requirements per metabolizable energy as determined by body weight gain and whole-body composition were significantly high in broiler-type males and low in Leghorn-type females. Since males have the potential of growing faster and becoming bigger, they are estimated to have amino acid requirements that are higher

than females for potential growth. Salehifar *et al*. (2012) also concluded that males have higher requirements for digestible amino acids than females. As a result, it is crucial that feeds are formulated to meet the needs of each sex, but this is not always the case as the two sexes are usually given a single feed which may result in the under- or oversupply of the amino acids required by a specific sex (Penz, 2002). The reason males have higher amino acid requirements than females is because of their faster growth rate and higher protein tissue deposition. There is no protein requirement for the retention of ash, lipid, and water (Gous, 1998).

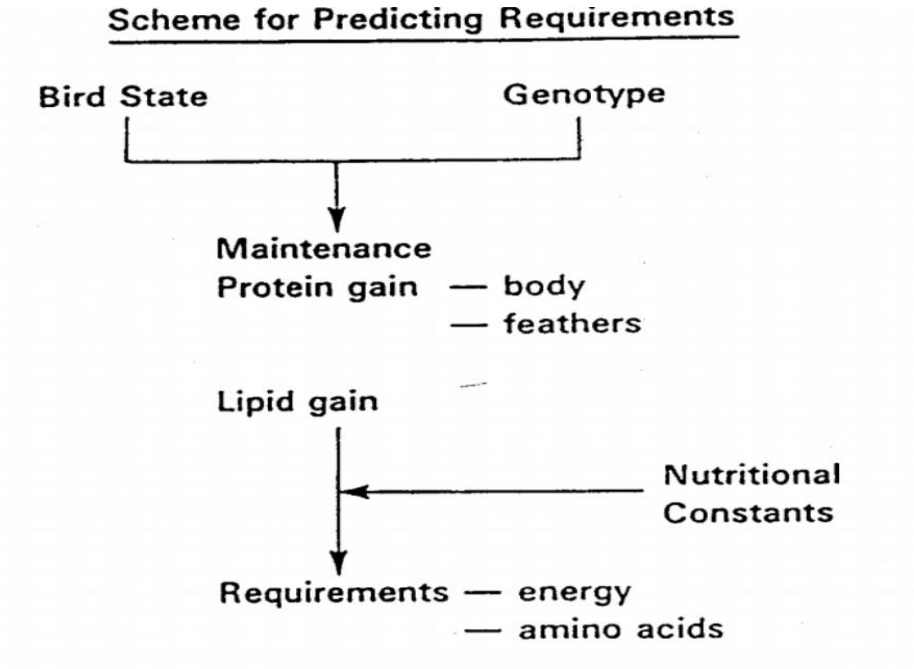

Figure 2.2 Scheme for predicting requirements, Source: Emmans (1987b)

Maertens *et al*. (1997) reported a significant interaction between daily weight gain and CP content in rabbits, which illustrated that post-weaning phase amino acid requirements of rabbits are dependent on age. Diets high in protein are required during the early growth stages due to the high requirements for crude protein (Kamran *et al*., 2008) and the low feed intake at that age. Young rabbits have relatively high protein and amino acid requirements due to intestinal growth, tissue accretion, and maintenance of the functionality of the intestinal mucosa (Trocino *et al.*, 2000).

Amino acid requirements in the feed of growing animals (g/kg feed) decrease with the increase in age and body weight because of the increase in feed intake as the animal grows. On the contrary, the daily amino acid requirement for growth will increase with the degree of maturity since the protein content of the non-lipid body rises with maturity level (Emmans, 1981).

According to Aviagen (2019), weekly feed intake increases with broiler age, consequently larger birds consume more feed to meet their nutritional needs. The genetic selection pressure in growing rabbits has resulted in fast-growing animals with an average daily weight gain of more than 45 g/day so the protein requirements of these animals would be higher than for those with a slower growth rate (Marín-García, 2021).

2.3 Ideal protein concept

2.3.1 Amino acid imbalances

Over the last four and six decades, the ideal protein concept has made significant progress in the fields of swine and poultry protein nutrition, resulting in the use of crystalline essential amino acids (EAAs) as a dietary supplement to increase pig and poultry growth, feed efficiency, and productivity (Boisen *et al* 2000; Baker, 2009; van Milgen and Dourmad 2015*)*. The ideal protein concept was suggested by Mitchell (1964) and is still applicable in some cases. However, these authors did not consider non-essential amino acids (NEAAs). The ARC (1981) was the first to implement the principle of ideal protein in pigs and this has gained substantial interest (Wang and Fuller, 1989, 1990; Fuller *et al*., 1989; Chung and Baker, 1992). In this concept, implemented by the ARC, arginine, histidine and NEAAs were ignored. This principle was based on determining the requirements of individual amino acids by evaluating the effect on performance when varying quantities of amino acids are added to the basal diet (Boisen *et al*., 2000).

The ideal protein concept is valid when all the first limiting amino acids are utilized with the same efficiency. For both maintenance and retention, the ideal balance of essential amino acids is identical (Whittemore, 1983). An ideal protein profile was described by Wang and Fuller (1989) as one that maintains fixed levels between lysine content and the minimal quantities of all other essential amino acids. Early researchers attempted to formulate purified diets for young chickens using the composition of EAAs in casein and chicken eggs as the experimental foundation, but this method failed because of the imbalances and excessive EAAs which contributed to poor weight gain and feed efficiency. Thus, the concept of ideal protein is not ideal in animal nutrition since it lacks the supplementation of NEAAs which must also be provided in the diet. Therefore, nutritionists should adopt optimum concentrations of all nutritionally and physiologically essential amino acids rather than focusing only on the ideal protein concept to help in the formulation of adequate low protein feeds that support animal production (Wu and Li, 2022).

Harper (1964) defined amino acid imbalance as an alteration in the amino acid sequence in the diet which results in the depression in food intake and growth that can be totally corrected by supplementation with the first-limiting amino acid or the use of low-protein diets. Reports on amino acid imbalances mainly focus on the negative impact on animal growth (Harper, 1964). According to research by Van Milgen and Dourmad (2015), an AA deficiency and oversupply results in reduced performance and increased nitrogen excretion, respectively. Decreased feed intake, abnormal behaviour, and impaired growth rate are caused by amino acid antagonism which results from amino acid imbalance where the amino acid supply is less than required (Wu, 2009). Amino acid antagonism is a result of interactions where the consumption of excessive quantities of one amino acid raises the need for another amino acid with a similar structure. Antagonism is however different from the amino acid imbalance because its effects are reversed by the supplementation of the amino acid that is structurally identical to the amino acid in excess, rather than supplementation of the limiting amino acid to the imbalanced diet. Antagonism between lysine and arginine was initially discovered in chickens. This antagonism is visible when the ratio of these two amino acids differs from what the animal requires.

Excess lysine dramatically decreases growth when arginine is marginally sufficient. This depressing effect may be reversed by the addition of arginine to the diet. Low valine levels in diets have been shown to reduce growth and feed intake and this effect is increased when leucine is in excess. This depression in growth and feed intake may be alleviated by the supplementation of valine to the imbalanced diet. Decreased valine levels resulted in decreased carcass protein and water contents but increased lipid contents (Meyer *et al*.,2017). Wiltafsky *et al*. (2010) also reported a depressed ADFI due to excessive leucine levels. Additionally, when the supply of amino acids exceeds the requirement, nutrients are wasted, resulting in nitrogen excretion, which has a detrimental effect on the environment and productive performance (Mordenti *et al.,*2003). According to Harper *et al*. (1970), increasing the protein content of a diet to a sufficient level by using natural sources of proteins will also increase the levels of limiting amino acids in the diet, which may lessen the severity of an amino acid imbalance.

2.4 Factors affecting amino acid utilization

An accurate estimation of the amino acid requirements of growing animals requires an accurate assessment of how efficiently these animals utilize amino acids (Reis *et al.,* 2018). Therefore, in order to make decisions and optimize dietary levels with the goal of minimizing the impacts

of both a limited and an excessive supply of nutrients, approaches that evaluate the effectiveness of nutrient utilization are required (Silva *et al.,* 2013). According to some authors, the efficiency of amino acid utilization for birds should be approximately 80% (Martin *et al.,* 1994). The maximum performance of an animal is also essentially achieved by its ability to efficiently utilize amino acids. The efficiency of amino acid utilization can be described as the fraction of the amino acids retained to amino acids digested.

Efficiency of amino acid utilization was studied in growing pigs by Heger *et al*. (2002). These authors estimated lysine, threonine, sulphur amino acids, and tryptophan to have efficiencies of ileal digestible amino acid utilization of 0.91, 0.83, 0.85, 0.85, 0.66, and 0.66 respectively. According to Nogueira *et al*. (2021), lysine utilization was 0.78, 0.76, and 0.78 in females and 0.8, 0.81, and 0.81 in males during the starter, grower, and finisher phases. These authors in turn found arginine utilization of 0.61, 0.63, and 0.61 in females, the values for males were 0.63, 0.63, and 0.62 in males during the starter, grower, and finisher phases, respectively. These findings also showed that during the starter, grower, and finisher stages, arginine utilization for females was 0.61, 0.63, and 0.61, while it was 0.63, 0.63, and 0.62 for males. This is depicted in Table 2.1

The efficiency with which amino acids are utilized for growth is determined by the environment, dietary content, genotype, and physiological condition. According to Harper (1984), the key biological factors influencing amino acid utilization are the relative quantities of amino acids in the diet and the total amounts of amino acids consumed. When Parks (1982) examined the effect of protein level on food intake and growth, he found that the protein retention efficiency increases as protein level increases until a point at which it starts to decline. In addition, as cited by Berhe (2008) numerous researchers suggest that as protein intake increases or energy declines, protein utilization does not remain constant but decreases progressively (ARC, 1981). The level of dietary protein and/or particular amino acid concentrations also affect lysine utilization. Lysine utilization efficiency is determined by the supply of other essential amino acids and the sum of non-essential amino acids if their ratios to lysine are above critical values. The efficiency of utilization of dietary protein is also affected by amino acid imbalance. As a result, Moughan (1991) linked reduced efficiency of protein utilization in pigs relatively to an imbalance of dietary amino acids. Furthermore, a lack of correct quantities of one amino acid in the diet will result in decreased efficiency of the other amino acid.

Table 2. 1 Efficiency of lysine and arginine utilization for male and female broiler chickens, and the equation of amino acid deposition (AAd) in the function of intake (AAi) adjusted to estimate the efficiency of utilization.

Age	Efficiency of Lysine Utilization		Efficiency of Arginine Utilization	
	Female	Male	Female	Male
1 to 14	0.78	0.8	0.61	0.63
15 to 28	0.76	0.81	0.63	0.63
29 to 42	0.78	0.81	0.61	0.62
Average	0.79		0.62	
p-value phase	0.954		0.963	
p-value sex	0.512		0.244	
Linear equation	AAd = 23.8(± 94.2) + 0.789(± 0.256) × AAi		AAd = −30.9(± 13.6) + 0.623(± 0.024) × AAi	

Source: Nogueira *et al*. (2021)

Comparative research has been done on various poultry genotypes to evaluate the efficiency of protein and energy utilization. It was found that the efficiency of protein utilization was the same in both fast-growing (broilers) and slow growing (cockerels) genotypes (Morris and Njuru, 1990). Genetic variation in chicks has also been reported to influence arginine utilization (Nesheim, 1968). In order to maximize protein utilization, the essential amino acid balance is crucial.

2.5 Factors influencing the response of growing rabbits to dietary protein

2.5.1 Effect of genotype

The concept of genetic potential has been extensively applied and it has been regarded as the performance level that can be attained in a non-limiting environment. The genotype and sex of an animal have a considerable effect on the response to dietary protein. In a study conducted by Kemp *et al.* (2005), it was shown that when two strains of commercial broilers were exposed to changes in the optimal dietary protein content, one strain responded to a decrease in protein by consuming more feed, while the other strain did the opposite. These responses are shown in Figure 2.3. This resulted to an obvious conclusion that the optimum feeds and feeding schedule of these two strains would differ.

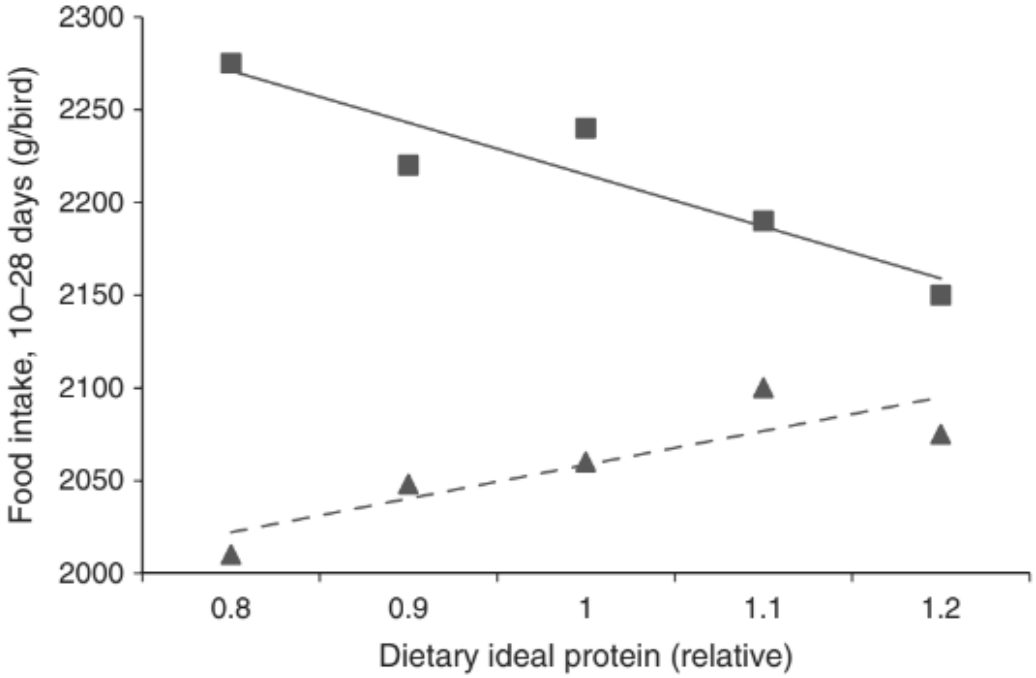

Figure 2.3 Changes in food intake in response to dietary ideal protein content, relative to the Aviagen (2009) recommendations, for Ross 308 broilers in two commercial broiler strains. (From Kemp *et al*., 2005).

Pigs with different genotypes may have varied lean growth potential, which influences their protein and amino acid (AA) requirements (Schinckel and de Lange, 1996). Pigs of present genotypes are considerably leaner than those of the past (Parson *et al*., 2005). In a study conducted by Marin-Garcia *et al*. (2020), where the aim of the study was to determine whether growing rabbits from three genetic lines with high growth rates will be able to sufficiently cover their protein requirements when feeding on diets with moderate protein content, it was found that all the three genetic types had different daily feed intakes, daily weight gains and feed conversion ratios. Previous studies investigated the responses of genetically lean and fat chickens to dietary protein and essential amino acids, where it was discovered that lean chickens were more sensitive to low amounts of dietary protein (Leclercq, 1983; Geraert *et al*., 1990) and sulfur-containing amino acids (Leclercq *et al*., 1993), arginine and lysine (Leclercq *et al*., 1994), and threonine (Alleman *et al*., 1999). Selection for fatter animals at a given age reduces Lipid: Protein ratio (LPRm) and may improve protein mature weight (Pm) to a lesser level.

The increase in the protein level of the diet led to a reduced feed intake of Peterson X Arbor Acres broiler males (Nakhata and Anderson, 1982; Parsons and Baker, 1982; Pesti and Fletcher, 1984). On the other hand, Ross X Ross 208 increased their feed intake which then

decreased when dietary protein increased (Smith et *al.*, 1998). Body protein, water, lipid, and ash all have potential growth rates which are Gompertz functions of time, i.e., each genotype shares the same rate of maturing parameter (Hancock *et al.*,1995).

2.5.2 Effect of environment

Environmental factors such as housing and temperature significantly impact growth and the feed conversion ratio of animals. In order for an animal to grow along its potential path, it needs an environment in which it can lose the heat produced as a result of reaching that path (Emmans,1989). Hence, a careful control of the physical microenvironment inside the animal production facility is required for the optimization of the production process (Reece and Lott, 1982 and Mitchell, 1985). If the environment is thermally neutral at all intakes, heat production on a feed of a particular composition will increase as feed intake increases. Animals that are not fed *ad libitum* lose less heat at low temperatures than those fed *ad libitum.* The other significant variables that influence the response of animals to heat stress are age, breed (size and feather cover) and nutrient density. In comparison with smaller pigs, heavier pigs tend to be more susceptible to hot temperatures (Quiniou *et al*., 2000).

The amount of heat that can be lost to the environment in poultry is primarily influenced by feather cover (Gous, 2018). However, the total heat that can be lost to the environment results from both the evaporative and non-evaporative heat loss components. Rabbits are susceptible to heat stress because their skin appears to lack sweat glands. Heat stress in rabbits has been shown to affect protein metabolism negatively (Marai *et al*., 2002) and results in reduced protein deposition (Temim *et al*., 2000). High temperature exposure has been linked to decreased growth rate and meat yield because animals consume less feed in an effort to reduce metabolic heat production and maintain body homeostasis. Additionally, according to Williams *et al*. (1993) and Dionissopoulos *et al*. (2001), environmental stress can markedly lower the rate of lean growth. An animal therefore needs to have the ability to lose heat to the environment for maximum performance. The combination of heat losses from the synthesis of protein and lipid, protein deamination, and maintenance is the total heat loss (Whittemore, 1983).

Various authors concluded that low protein diets in broilers reared under heat-stress environments are beneficial. In contrast, Fulan *et al.* (2004) have shown that low-protein diets have a negative impact on broiler performance under high environmental temperatures. This is because under conditions of heat stress, reduced food intake combined with a low protein diet

can result in amino acid deficiencies. The reduced growth rate in hot environments can lower pig carcass weight which can also lead to a decrease in revenue of pork (Liu *et al*., 2021). It is therefore crucial to provide animals with a thermally neutral environment that will allow them to lose heat to reach their potential growth path.

2.6. Discussion

Given that there is little to no literature on the response of growing rabbits to balanced dietary protein, the focus of this review was to highlight the significance of measuring the response of growing rabbits to dietary protein by using data of measured responses to dietary protein of different growing animals from which the optimum dietary protein level to include in feeds was determined for maximum productivity and profitability. Breed, sex, dietary protein content, environment, age and physiological condition of an animal were the key factors highlighted as influencing this response.

The theory proposed by Emmans (1989), states that a growing animal will always seek to grow along its potential growth path, but this is not always achievable. Being able to achieve this growth potential depends on the feed offered and the environment in which the animal is growing. Some previous studies have shown that different breeds or strains respond differently to dietary protein, implying that the feeding regime of these breeds must be considered separately for efficient utilization and optimum production. It is in this regard that two different rabbit breeds were used in this study to measure whether their responses to dietary protein differed, and to what extent. It is expected that rabbits of different genotypes will respond differently to balanced dietary protein levels. This expectation is mainly rooted in the fact that the genetic background of an animal influences the rate of occurrence of biological processes at a genetic level in the animal body. Therefore, the amounts of nutrients required to meet the needs of each genotype for growth rate will be influenced by this rate.

It is also evident that different sexes respond differently to dietary protein due to the differences in their protein needs (i.e., their potential growth rates). Males have a higher growth rate and protein deposition than females and this also results in their having higher amino acid requirements than females. This higher protein accretion is also due to the physiological differences that exist among the sexes. Males have been proven to have inherent bigger weights than females.

Age has also been considered as a crucial factor influencing the response to dietary protein. There is evidence that pigs and poultry prefer high protein diets in the early stages of growth

then switch to the low protein diets as they grow. This may be associated with the requirements of growing animals for protein which decrease with age.

2.7 Conclusion

The literature reviewed above focused mostly on how growing pigs and broilers respond to balanced dietary protein. There is however lack of information about how growing rabbits respond to balanced dietary protein levels. And this information is crucial when modelling feed intake, growth rate and carcass composition of growing rabbits so that decisions are made as to which feeding programs to use for each breed considering the economic and biological aspects of production.

Chapter 3

Response in growth performance of growing rabbits to balanced dietary protein

Abstract

Measuring the response of growing rabbits to various dietary protein levels can significantly contribute to the development of rabbit models. This study was conducted to measure the response in feed intake, body weight gain and final body weights of New Zealand White (NZW) and Californian (CAL) growing rabbits to balanced dietary protein. Equal numbers of males and females were used within each breed (12 CAL males and 12 CAL females, and 24 NZW males and 24 NZW females). The rabbits were offered the experimental diets for a period of eight weeks from a weaning age of 5-6 weeks. The feeding trial was divided into two periods, period 1 (1-28d) and period 2 (29-56d). Two balanced protein basal feeds, one high in protein (213g/kg) and other low in protein (126g/kg) were formulated using a well-balanced combination of amino acids to contain similar amounts of metabolizable energy and important minerals. Four additional protein levels were produced by blending the basal diets resulting in a total of six dietary protein levels (126, 143, 161, 178, 196 and 213g/kg). Feed intake and body weight of each rabbit were measured weekly up to 56d. Feed intake and final body weight were linearly related to balanced dietary protein content ($P < 0.05$), with highest feed intake being observed on the NZW males. FCE was not influenced by the dietary protein ($P > 0.05$). Results of this experiment also revealed that the NZW rabbits had the highest body weight gain and were more efficient in converting feed to weight gain than the CAL rabbits. Further research is therefore needed to corroborate the findings of this study for an accurate estimation of balanced dietary protein content to include in growing rabbit diets of different breeds and sexes for maximum performance and profitability.

Key words: growing rabbit, feed intake, growth rate, balanced dietary protein, body weight

3.1 Introduction

Experiments aimed at measuring and predicting the response of broilers and pigs to dietary protein have received considerable attention (Kassim and Suwanpradit, 1996; Widyaratne and Drew, 2011; Srilatha *et al*., 2018; Ojederiran *et al*., 2021), the main focus being to investigate performance parameters such as body weight gain, feed intake and feed conversion efficiency.

Research focusing on growing rabbits has received far less attention in this regard in comparison to other animals. This could be due to the fact that rabbits have been neglected as an important species in the Agricultural industry and rather been considered as a pet bunny, yet it possesses positive traits which are beneficial to human health. As there is much value in knowing how growing animals respond in growth performance to dietary protein, this project was designed to address this issue by measuring the response of two breeds of growing rabbit to dietary protein.

Dietary protein content influences feed intake, body weight gain, feed conversion efficiency, and, consequently, the optimal economic level of dietary protein would differ depending on the cost of protein-containing ingredients and the value of the product sold (Azevedo *et al*. 2021b). Response of broilers to balanced dietary protein have recently been measured by Azevedo *et al*. (2021a). These authors concluded that feed intake increased with decreasing dietary protein levels and then dramatically decreased at the lowest protein levels for the two strains and sexes in all feeding phases. Additionally, it has also been demonstrated that the performance of pigs is influenced by the dietary protein content. Jansman *et al*. (2016) reported that the performance of weaned pigs decreased dramatically when crude protein was reduced below 160 g/kg. Therefore, knowledge of how growing rabbits respond to various dietary protein levels can be applied to accurately determine the optimum protein content to include in feeds for maximum profitability and cost margins, and consequently, growth rate. Given that animal feed cost makes up about 60-70 percent of the total cost of animal production (Abdu *et al*., 2019), feed cost is a key factor that needs to be considered in a production enterprise.

It is well known that researchers and farmers are interested in altering dietary nutritional concentrations of the feed to meet the dietary needs of growing animals according to breed and sex while also aiming for maximum productivity with the least potential production costs. Consequently, this study will be able to determine the required amount of dietary protein that will enable the rabbit to grow at its potential, so that experiments designed to measure the potential growth of rabbits would benefit from the results of this trial. The current study was then designed to measure the response of growing rabbits of two breeds and sexes in growth performance to various levels of balanced dietary protein so as to be able to determine the optimum economic amounts of dietary protein levels to be included in feeds.

3.2 Materials and methods

3.2.1 Ethical consideration

All experimental protocols were approved by the Animal Ethics Committee of the University of KwaZulu-Natal, South Africa (ethical clearance, ref no; AREC/00002324/2021).

3.2.2 Study Site

The trial was conducted at Ukulinga Research Farm in Pietermaritzburg, KwaZulu-Natal (KZN), Republic of South Africa (RSA). The farm is located under the coordinate: latitude 28° 24′ E and longitude 30° 24′ S at an altitude of 775 meters above sea level. The farm is dominated by trees such as *Acacia karroo, Acacia nilotica,* and *Acacia sieberiana*. The climate of the area is characterized by a mean annual maximum and minimum temperature of 27.7°C and 8.9 °C, with a mean annual rainfall of 735 mm occurring in the summer season.

3.2.3 Experimental design

A total of 72 weaned rabbits, 48 New Zealand White (NZW) and 24 Californian (CAL), between 5 and 6 weeks of age, were randomly distributed among six balanced dietary protein levels following a 2 x 2 x 6 factorial arrangement with equal numbers of males and females within each breed. These rabbits were sourced from a local breeder, Future Farmers, based in Howick, which is 48km from the farm, and were transported in the morning before the sunrise in well-ventilated travelling cages at the back of a well-sealed aerated bakkie. Rabbits were housed singly, this being the experimental unit. The rabbits were subjected to experimental diets for 56 days.

3.2.4 Animal housing and management

Each rabbit was tattooed, and its body weight was recorded at the start of the trial. The rabbits were randomly allocated, individually, to 72 wire cages inside the rabbit house. The wire cages ensured a sufficient ventilation to allow good air movement. Wood shavings were spread underneath the cages to absorb urine. Each cage was equipped with a feeder suspended at a reasonable height in front of the cage and 2-3 nipple drinkers inside each cage. The temperature was controlled by curtain adjustment in the rabbit house.

3.2.5 Experimental diets

The feeding program of the rabbits was divided into two four-week phases starting at weaning, with dietary protein levels being reduced in each subsequent period but with the same relative difference between levels being maintained. The two basal feeds were formulated to contain 4.9 and 8.1 g digestible lysine (dLys)/kg, respectively, each feed containing 10.0 MJ DE/kg

(Table 3.1). These basal feeds were formulated using the WinFeed formulation software to produce a high and low protein basal feed, the experimental feeds being produced using the dilution technique of Fisher and Morris (1970) in which a low and high protein basal diets (table 3.2) are appropriately blended to produce the range of experimental feeds containing protein levels of 128, 143, 161, 178, 196 and 213g/kg. After blending, all dietary treatments were sampled for Crude Protein, Crude fiber and Gross Energy analysis (AOAC, 942.05). The amino acid levels used in the feeding program, and the balance between amino acids, which were the same in both basal feeds, are based on those recommended by De Blas *et al*. (1998) as were the major and minor mineral contents, and energy. All feeds were pelleted before being offered to the rabbits. Feed and water were offered at *ad libitum* to the rabbits immediately upon arrival until slaughter. The rabbits were monitored twice a day, in the morning and afternoon to ensure that feed and clean water is available at all times.

3.2.6 Chemical composition of experimental diets

Chemical analysis (AOAC, 1990) of the experimental diets was performed in duplicates at the University of KwaZulu-Natal, Animal and Poultry Science Laboratory, Pietermaritzburg, South Africa. The chemical analysis is shown in tables 3.3 and 3.4 below.

3.2.6.1 Gross energy

A bomb calorimeter was used for the gross energy analyses. Megajoules (MJ) per kg were used to express the gross energy values. Retch ultra-centrifugal mill was used to grind the experimental pelleted diets through 1mm sieve. On each dietary treatment, duplicate analyses were carried out from which averages were determined.

3.2.6.2 Crude Protein

Nitrogen content was measured using the Dumas Combustion method in a Leco Truspec Nitrogen Analyzer, St. Joseph, MI, USA, by method 990.3(AOAC, 1990). Crude protein (CP) content was estimated using the formula: N× 6.25.

3.2.6.3 Crude Fiber

Crude fiber analysis was carried out using filter bag technique by. Following drying, a sample was heated in weak sulfuric acid (1.25% H2SO4) and filtered. The residue was filtered after being heated in a weak alkali (1.25% NaOH), and the remaining residue was then dried and ashed. The difference between the filtered dried sample and ash is crude fiber.

3.2.7 Growth performance measurements

3.2.7.1 Feed Intake

Feed for each rabbit was weighed into a container at the start of each week and allocated from there to the feeder each day, ensuring that there was always feed available in the feeder. A micro A12E platform scale was used to weight the feed. The feed remaining at the end of the week was weighed back to calculate food intake, and the container was then re-filled and weighed. Feed intake was calculated as the initial feed weighed into the container minus the feed remaining in the feeder and the container. An effort was made to collect feed spillage daily, weighed and recorded.

Table 3. 1 Ingredients and nutrient composition in the low and high basal protein feeds

Ingredient	Low protein basal (kg)	High protein basal (kg)
Barley	114	68.3
Oats	150	50
Wheat bran	62.7	-
Molasses	0.75	2.5
Sunflower hulls	-	60
Soybean 46	-	67.4
Sunflower 37	-	82.3
Lucerne meal 15%	165	165
Limestone	2.4	1.3
Salt	1.7	1.85
Monocalcium phosphate	0.1	0.1
Oil sunflower	0	4.05
Robenidine	0.05	0.05
L-lysine HCL	0.25	0.15
L-threonine	0.1	0.5
DL Methionine	0.3	0.75
Vit+min premix	0.75	0.75
Calculated composition (g/kg)		
Crude Protein (N × 6.25)	117	172
Crude Fiber	9.4	12.6
Gross Energy (MJ/kg)	17.1	17.1
ME (MJ/kg)	14	14

ME-Metabolizable energy

Table 3. 2 Proportions of high and low protein basal feeds used for each dietary treatment and feeding period

Protein	Period 1		Period 2	
	HP	LP	HP	LP
1	20	80	0	100
2	36	64	16	84
3	52	48	32	68
4	68	32	48	52
5	84	16	64	36
6	100	0	80	20

Table 3. 3 Proximate chemical analysis of the two basal feeds for period 1 (g/kg)

Experimental diets	Crude protein g/kg	Crude Fiber g/kg	Gross Energy (MJ/kg)	ME[1] (MJ/kg)
1	127	10.1	17.5	14.4
6	170	10.0	17.0	13.9

[1] Calculated as GE * 0.82

Table 3. 4 Proximate chemical analysis of the two basal feeds for period 2 (g/kg)

Experimental diets	Crude Protein g/kg	Crude Fiber g/kg	Gross Energy (MJ/KJ)	ME (MJ/KJ)
1	117	8.9	17.2	14.1
6	159	12.2	17.1	14.0

[1] Calculated as GE * 0.82

3.2.7.2 Body weight

Rabbits were individually weighed on a weekly basis by placing them inside a box on a pan-scale to determine body weight gain. Body weight gain was calculated as the final body weight minus the initial body weight of the rabbit. Daily gains were determined by dividing the weekly body weight gains by seven (days).

$$ADG = \frac{Body\ weight\ gain}{total\ no\ of\ days\ of\ the\ trial\ (56)}$$

3.2.7.3 Feed conversion efficiency

Feed conversion efficiency (FCE) was determined by dividing body weight (g) gained by the animal by feed intake (kg). The formula for the determination of the FCE is displayed below.

$$FCE = \frac{g\ gain}{kg\ food}$$

3.2.8 Statistical analysis

The trial was designed as a response to six protein levels with two factors (two breeds and two sexes). Performance (feed intake, body weight gain and FCE) was analysed using (GenStat 20th edition, VSN International, 2022). Analysis of variance was used to determine treatment means, and appropriate regression analysis were used to describe the responses to dietary protein content in the variables of interest. Tukey's significant difference test was used to compare means. Main effects and two-way interactions between strain and sex were compared using linear regression with groups (GenStat 20th edition, VSN International, 2022). Significance was determined at $P<0.05$. Linear and exponential regression models were used to describe the responses to dietary protein.

Linear model: $Y = a \pm bx$

Where:

Y = Variate being regressed

a = Constant term

b = Regression coefficient

x = dietary protein level

Exponential model: y = A + B* (R**X)

Where:

A + B is the y-intercept

R is the exponential base

X is the level of the balanced protein

3.3 Results

3.3.1 Growth performance parameters

The mean performance (feed intake (g/d), body weight gain (g/d), and FCE (g gain/kg feed)) of two rabbit breeds fed diets with six dietary protein levels for the 56-d period is given in Tables 3.5 and 3.6, respectively. Appropriate regression models were fitted to the data. Different models, including exponential, quadratic, and linear, were used. The model with the best statistical fit was selected. Quadratic coefficients are not presented as they did not show a significant difference ($P > 0.05$) in all response variables. Simple linear regression with groups was used, groups being breed and sex. Exponential regression best described the response in body weight gain, with values for coefficients A, B and C differing, but with the same R coefficient for both breeds and sex combinations. Exponential response in body weight gain is demonstrated in figure 3.2. The simple linear regression coefficients are presented in Tables 3.7 to 3.9. Linear responses are also demonstrated graphically in figures 3.1 and 3.3. Feed intake was linearly related to dietary protein levels. The constant term differed among the breeds with the highest feed intake being observed on the NZW breed ($P < 0.05$). The NZW rabbits had higher body weight gain and were more efficient in converting feed into body weight gain than the CAL rabbits. Significant two and three-way interactions ($P < 0.05$) between (breed x sex), and (dietary protein, breed, and sex) were detected in the cumulative feed intake of females for the entire 56 d period. The average weaning weights were respectively 1470 g for NZW and 2179 g for CAL rabbits. The final body weight of the two strains showed a significant response to dietary protein ($P < 0.05$). A significant interaction between (breed x sex) was observed in final body weight ($P < 0.05$) as well as between (protein x breed x sex) females in the final body weight ($P < 0.05$). NZW rabbit females and males had the highest feed intake on the lowest and highest dietary protein level, respectively, whereas CAL female and male rabbits had the highest feed intake on the highest and second highest dietary protein levels, respectively. Females of the two breeds responded differently to the dietary protein content in final weight ($P < 0.05$). There was no significant effect of dietary protein levels on the feed conversion efficiency ($P > 0.05$).

Table 3. 5 Mean feed intake (g/d), final body weight (g) and feed conversion efficiency (FCE, g gain/kg feed) of female and male New Zealand White (NZW) and Californian (CAL) rabbits fed a range of dietary protein levels for a period of eight weeks.

	Feed intake, g/d				Final body weight, g				FCE (g gain/kg feed)			
	NZW		CAL		NZW		CAL		NZW		CAL	
Protein g/kg	F	M	F	M	F	M	F	M	F	M	F	M
126	166	180	138	144	2902	2800	2609	2541	217	191	151	136
143	153	169	184	154	2723	2756	3046	2560	204	208	156	143
161	164	176	169	157	2758	2792	3352	2715	222	205	112	128
178	151	161	170	159	2718	2771	3322	3208	241	215	136	86
196	139	177	184	173	2598	2852	3356	3002	232	192	149	120
213	149	185	197	164	2632	2935	3291	3329	188	197	140	147
MEAN	154	175	174	159	2721	2818	3163	2392	222	211	138	111

	SEM[1]	P-value	SEM	P-value	SEM	P-value
Protein	6.6	0.5	46.9	<.001	10.7	0.327
Breed	4	0.57	27	<.001	6.2	<.001
Sex	3.8	0.037	27	0.023	6.2	0.029

[1] SEM: Standard error of mean

Table 3. 6 Mean daily body weight gain of the NZW and CAL rabbits fed dietary protein levels over 56 d period.

	Body weight gain (g/d)			
	NZW		CAL	
Protein g/kg	F	M	F	M
126	36	35	21	20
143	31	35	29	22
161	36	36	19	20
178	36	35	23	14
196	33	34	27	21
213	28	36	28	25
MEAN	33	35	25	20

	SEM	P-value
Protein	2.298	0.998
Breed	1.387	<0.001
Sex	1.328	0.792

SEM, Standard error of mean

Table 3. 7 Linear regression coefficients describing feed intake (g/d) of male and female NZW and CAL rabbits over 56d period

Parameter	Estimate	SE	t (60)	t pr.
Constant	91.8	25.9	3.54	<.001
PROTEIN	0.481	0.151	3.20	0.002
BR x Sex CM	22.6	36.6	0.62	0.540
BR x Sex NF	100.6	31.7	3.17	0.002
BR x Sex NM	71.1	32.6	2.18	0.033
PROTEIN.BR x Sex CM	-0.223	0.213	-1.05	0.300
PROTEIN.BR x Sex NF	-0.707	0.184	-3.84	<.001
PROTEIN.BR x Sex NM	-0.0409	0.189	-2.16	0.035
R^2 = 0.57				

Abbreviation: SE, standard error; t, test statistic, t. pr, t-probability; Br, Breed; CM, CAL male; CF, CAL female; NF, NZW female; NM, NZW male.

Factor Reference level:

BR x Sex CF

Table 3. 8 Exponential regression coefficients describing the body weight gain (g/d) of male and female NZW and CAL rabbits over 56 d period

Coefficient	NZW		CAL	
	F	M	F	M
A	34.89	35.02	23.51	18.87
B	-4.749E-08	7.684E-09	3.214E-08	4.076E-08
R	1.0922			
	R^2 = 0.38			

Table 3. 9 Linear regression coefficients describing the final body weight of male and female NZW and CAL rabbits over 56 d period

Parameter	Estimate	s. e	t (60)	t pr.
Constant	2326	529	4.40	<.001
PROTEIN	7.48	3.07	2.43	0.018
BR x Sex CM	-412	748	-0.55	0.584
BR x Sex NF	1732	648	2.67	0.010
BR x Sex NM	885	655	1.33	0.188
PROTEIN.BR x Sex CM	-0.17	4.35	-0.04	0.969
PROTEIN.BR x Sex NF	-11.64	3.76	-3.09	0.003
PROTEIN.BR x Sex NM	-5.97	3.86	-1.55	0.127
R^2 = 0.57				

Factor Reference level

BR x Sex CF

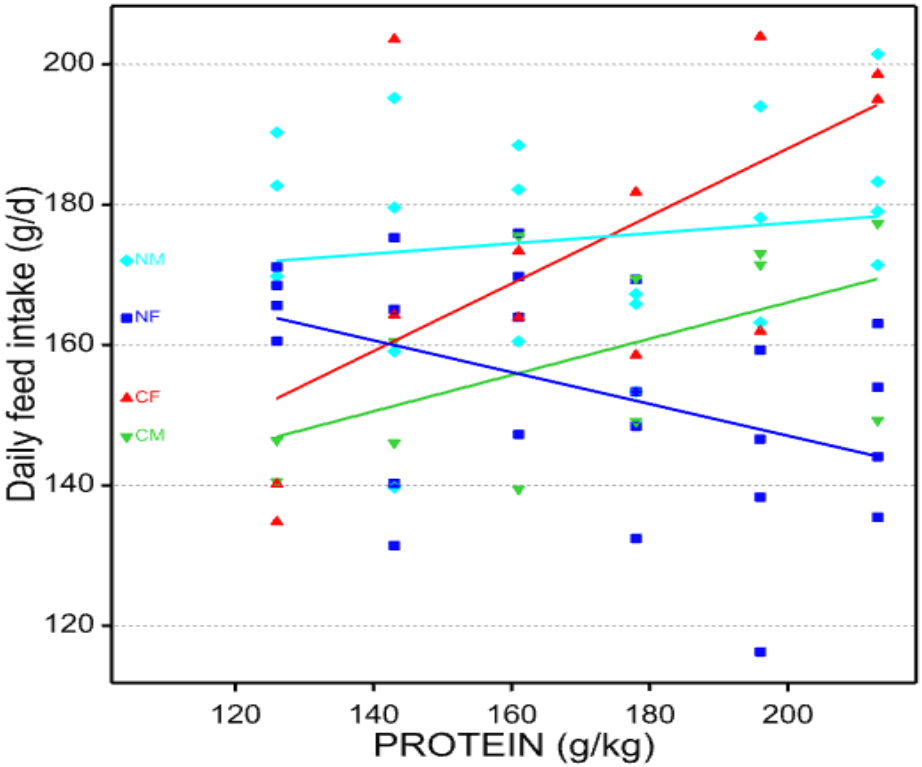

Figure 3.1 Fitted and observed relationship between dietary protein content and feed intake of CAL and NZW male and female rabbits. NF (New Zealand White female rabbits), NM (New Zealand White male rabbits), **CF (**Californian female rabbits**),** CM (Californian male rabbits).

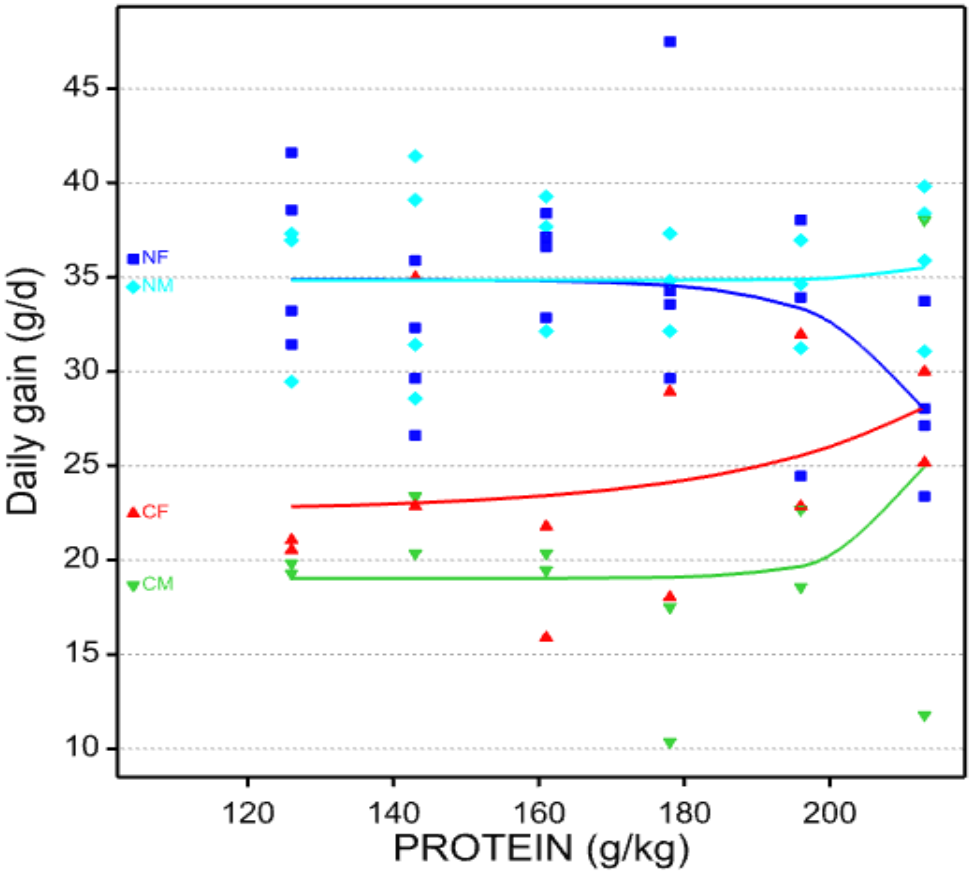

Figure 3.2 Fitted and observed relationship between dietary protein content and daily gains of the CAL and NZW male and female rabbits.

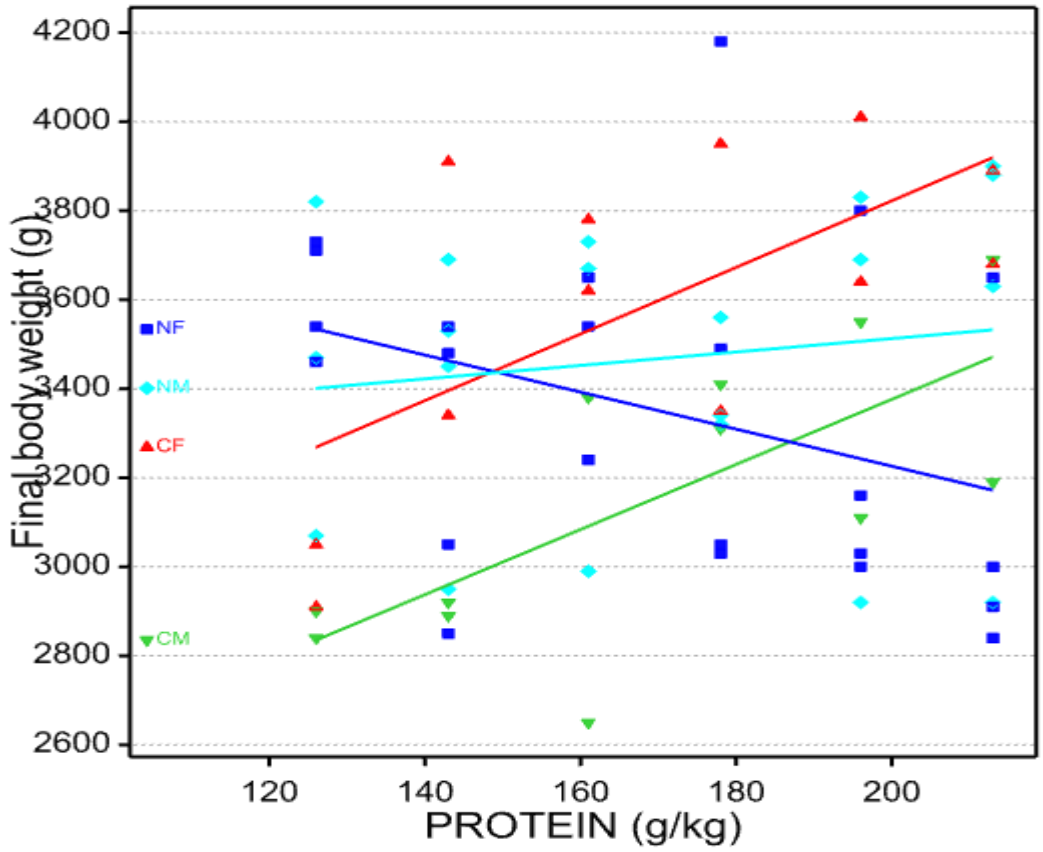

Figure 3.3 Fitted and observed relationship between dietary protein content and final weight of CAL and NZW male and female rabbits.

3.4 Discussion

Rabbits are an advantageous and affordable source of protein to meet the increasing human demands in developing countries (Nehad *et al*., 2009). In general, the costly protein-containing feed materials used in rabbit diets pose a potential challenge for nutritionists. However, knowledge of responses of different rabbit breeds and sexes to balanced dietary protein can be used to determine the optimum dietary protein levels to include in diets for maximum performance and profitability. The current study was therefore designed to measure the response of two growing rabbit breeds to dietary balanced protein. Due to the varying inherent potential of different genotypes and the different nutrient requirements that males and females have, it was expected that the two breeds and sexes would respond differently to the dietary protein levels. In addition to differences in animal genotypes, the response of the overall population to a given diet and environment is also influenced by variations in nutrient quantity and quality, environmental changes, and sample size (Ferguson *et al*., 1997).

Based on the previous findings (Kemp *et al.*, 2005; Berhe and Gous, 2008; Azedevero *et al*.,2021a) feed intake was expected to increase as the dietary protein content was reduced, followed by a decrease in feed intake at the lowest protein levels. However, in the current study this was not the case. Results on the two breeds and sexes did not show any clear pattern in their feed intake. This proves that there were significant differences in the responses to feed intake due to variations in feed intake between the two breeds and sexes or even within the different sexes of the same breed. The variation in feed intake has also been found to be the first factor which results to variation in growth performance. Besides the different nutrient requirements that different breeds and sexes may possess which may in turn influence the variation in feed intake, there are other factors which may greatly influence this variable. Nyachothi *et al*. (2004) revealed that gut capacity, bulk density of the feed, and ambient temperature are other significant factors influencing the feed intake of growing animals.

Feed intake may be restricted by gut capacity, particularly in young animals subjected to diets of low energy density. Water holding capacity is primarily influenced by the type and amount of dietary fiber. The ability of an animal to lose heat to the environment also influences feed intake and this ability has been discovered to be influenced by the feather cover in broilers.

Regarding the effect of gender and breed on feed intake, the sexes of different breeds responded differently to the dietary protein content ($P < 0.05$) throughout the 56d period. This varying

response to balanced dietary was also observed by Azevedo *et al*. (2021) in male and female Cobb and Ross strains throughout the 56d experiment. The CAL and NZW female rabbits responded differently to the dietary balanced protein with the highest feed intake being observed on the highest protein level in CAL female rabbits whereas the NZW females had their highest feed intake on the lowest protein level. A highly significant ($P < 0.001$) protein x breed x sex (females) interaction was also observed in these two sexes of these breeds showing this varying response. Another significant interaction was detected between the female CAL and male NZW rabbits ($P < 0.05$).

The interaction observed on the females of the two breeds was due to the change in feed intake between the two breeds as the female NZW breed increased feed intake with decreasing protein content while the CAL breed of both sexes decreased feed intake with decreasing protein content. This observed interaction could also imply that the feed intake of these breeds was dependent on dietary protein levels. Al-Sagheer *et a*l. (2020) reported that NZW-growing rabbits showed an increase in feed intake as a result of the decline in dietary protein level, moreover this is what would be expected in this study and this expectation is solely based on the theory of feed intake by (Emmans and Fisher 1986) which states that as the nutrient content in a diet is decreased, the animal will consume more of the provided feed in an attempt of meeting its nutritional requirements. This theory has also been supported by studies in broilers (Burnham *et al*., 1992) and laying hens (Gous *et al*., 1987) as both of these species increased feed intake in response to a reduction in the limiting nutrient in feed.

The observed interaction in NZW and CAL breeds could also suggest that the two breeds had different protein needs and that in turn affected performance. Breed of rabbits has been considered one of the major factors which affect the overall performance of the rabbits, particularly growth performance (Ologbose, 2017).

Lowest feed intakes were observed on the lowest dietary protein content (126 g/kg) in both male and female CAL rabbits, while on the contrary, the lowest feed intakes of the female and male NZW rabbits were observed on the highest protein level (213 g/kg) and 178 g/kg dietary protein content, respectively. This observation would simply imply that these two breeds have different protein needs for optimum performance. The obtained results are in line with those of Indarsih and Tamsil (2012), who concluded that different broiler genotypes had varying nutritional needs as a result of their different performance responses. New Zealand White male rabbits had higher feed intakes than the NZW females in all the dietary protein levels whereas the opposite was observed in the CAL rabbits.

The NZW rabbit females and males had the highest feed intake on the lowest and highest dietary protein level, respectively, whereas CAL female and male rabbits had the highest feed intake on the highest and second highest dietary protein levels, respectively. The difference in the feed intake response of the NZW sexes implies that the optimum dietary protein levels to include in feeds of these different sexes within this breed would differ to meet their requirements. The same response exhibited by the female and male CAL rabbits would imply that the rabbits of this breed do not have different protein requirements for growth as they both increased their protein intake with increasing protein content.

New Zealand White rabbits had higher body weight gain than the CAL rabbits with NZW males showing the highest gains despite their lower weaning weights. The variations in weight gain could be due to differences in breed type. The other factor that influences weaning weights of rabbits is litter size. Poigner *et al*. (2000) concluded that rabbit kits from larger litters tend to have lower weights at weaning than those from small litters. This was related to their capacity to gain weight depending on the quantity of milk they consume.

Final body weight was significantly influenced by the dietary protein ($P < 0.05$). Significant two- and three-way interactions (breed x sex), and (dietary protein x breed and sex) were observed on the final body weight of females of the two breeds ($P < 0.05$), respectively. The highest final body weight was observed on the NZW female rabbits receiving the lowest dietary while the highest body weight of the female CAL rabbits was observed on the highest dietary protein level. No significant interactions were observed between the NZW males and the reference breed (Californian female). Males have the potential to grow faster and to a larger size than females and this was evident in the NZW breed as males had higher body weights than females in all dietary protein levels. However, this was not the case with the CAL males as the females were observed higher body weights on all dietary protein levels except on the highest dietary protein content. The varying responses in final body weight of these breeds is probably related to their genetic background.

Dietary protein did not influence the FCE of the two breeds. NZW breed had numerically higher mean FCEs than the CAL breed with the highest FCE being observed in the NZW females. Only the NZW female rabbits had their lowest FCE on the highest dietary protein level. However, the low FCEs on the highest dietary protein level may have resulted from the possibility that at the highest dietary protein level, the rabbits may have consumed excessive protein which would have decreased their ability to efficiently utilize protein for protein gain.

There is however variation that exists between animals of the same sex and breed. One of the commonly known variations that exist between animals is the varying weaning weights. This variation however was evident in the animals used in this study. Different responses of different breeds and sexes could have been brought by this variation. Large variation in animals within the same dietary treatments was also evident as these variations within these animals resulted in no clear trends in feed intake. As a result of these variations which may have led to variations in feed intakes, conclusions that state that the different breeds responded differently to balanced dietary protein levels can be deducted.

Some animals of the same flock have higher weaning weights than others and this variable influences the growth rate and mature weight. In this study, the CAL rabbits comprised of higher weaning weights than the NZW rabbits and weaning weights are known to have an influence on the final weights. Therefore, the CAL rabbits were expected to have higher final weights than the NZW breed, however the results differed in the current study. Higher birth weights in rabbits have been shown to influence weaning weights and this relationship is influenced by environment, genetics and the level of feeding (Lukefahr *et al*., 1984). The highest final weights of the female CAL rabbits may be a result of their high weaning weights. Influence of weaning weights on growth performance traits of rabbits was studied by Oke *et al*. (2011). These authors found that Chinchilla rabbits with high weaning weights had higher final feed intakes, feed conversion efficiencies and body weight gains. In contrary to these findings, the NZW rabbits used in this study had higher cumulative feed intakes, body weight gains and consequently higher FCEs, regardless of their lower weaning weights. This might be due to their genetic potential as it is known to their faster growth rate (Brahmantiyo *et al*., 2018).

Precision livestock nutrition starts with matching nutrient supply with animal nutrient needs. It is, therefore, common knowledge that the nutritional needs of growing animals change as they grow. As a result, it may not be feasible to meet the needs of these animals by subjecting them to a single diet over the entire production period. According to some choice-feeding studies conducted on broiler and pig species, it is evident that these animals can choose between various diets for an intake of nutrients that meet their needs as they turn to select the high protein diets in their early growth stages and then switch to the low protein diets as they grow (Gous, 1990; Kyriazakis *et al.,* 1990; Abdella, 2005). Additionally, young rabbits may also consume more of a higher protein diet after weaning since their needs for amino acids and protein are higher at this age, but as they mature, they consume more of the medium protein

diet due to a decline in their protein needs (Xiccato and Trocino, 2011). In a study by Maertens *et al*. (1997), it was also concluded that in order to meet the protein needs of growing rabbits, the dietary protein levels need to be taken into consideration for different ages during growth.

Except for the lowering of protein level during the growing phase to meet the requirements of the growing animals, there are other benefits of this approach. One of the widely known benefits is the reduction of the N-excretion to the environment. Reduction of protein content in animal diets has also been emphasized to be beneficial in high environmental temperatures by increasing the feed intake, hence performance (Waldroup, 1982). Performance in broilers exposed to heat was also found to be improved by the reduced protein diet and ideal protein concept (Faria Filho *et al*., 2007).

3.5 Conclusions

In summary, it can be concluded that the level of dietary protein, breed, and sex have a significant effect on the response in growth performance of growing rabbits based on the significant interactions between dietary protein level, breed, and sex in feed intake and final body weight. The response to balanced dietary protein content differed among the different breeds in this study. However, dietary protein content resulted to a similar response in the FCEs of the breeds. Further studies are therefore needed to corroborate these findings for an accurate estimation of balanced dietary protein content to include in rabbit diets of different breeds and sexes for maximum performance and profitability.

Chapter 4

Response of Growing Rabbit in Carcass Composition to Balanced Dietary Protein

Abstract

The basis for estimating the nutritional requirements of growing animals can be found in the understanding of the changes in body composition that occur throughout growth. This study aimed at measuring the response to dietary protein in carcass composition, namely moisture, ash, lipid and protein of two rabbit breeds and sexes. A total of 8 New Zealand White rabbits were initially sacrificed before the commencement of the feeding trial to estimate the initial carcass composition of the remaining NZW rabbits offered the dietary protein treatments. Dietary protein concentrations in experimental diets (g/kg) were 126, 143, 161, 178, 196 and 213 and were fed for a period of 8 weeks. The rabbits were offered the experimental diets in two phases from 1 to 28 and 29 to 56 d. At the end of the feeding trial, a total of forty-eight rabbits (24 NZW and 24 CAL comprising equal numbers of males and females within each breed) were sampled for carcass analysis. Standard methods were used to determine the chemical composition of the rabbits. Moisture, ash, lipid, and protein did not show a significant response to balanced dietary protein ($P > 0.05$). CAL breed exhibited higher ash, lipid and protein contents than the NZW breed ($P > 0.05$). Highest moisture contents were found on the NZW rabbits. Increasing the range of dietary protein levels may result to a more significant variation in how the rabbits respond to balanced dietary protein, thereby providing a more precise understanding of growing rabbit response to protein levels.

Key words: body composition, lipid, genotype, protein body content, moisture content

4.1 Introduction

Along with growth performance responses, dietary protein concentration has been shown to have a marked effect on the carcass composition, particularly body fat and protein contents. According to Gheorghe *et al*. (2016), dietary crude protein content reduction results in decreased carcass protein and increased fat contents. Furthermore, Eits *et al*. (2003) also demonstrated that broiler carcass composition is influenced by previous protein nutrition and sex and as a consequence, it was recommended by these researchers that the protein levels in diets for grower and finisher phases should be optimized together rather than separately.

Genetics, age, sex, body weight, nutrition (particularly protein and energy), and environmental factors all have a significant influence the carcass composition of an animal.

Different genotypes may have different protein and lipid growth potentials, which would in turn influence their amino acid and protein requirements (Schinckel, 1996). There are two significant ways in which genotypes differ. Firstly, is how they will be of a certain size (mature protein size) and composition (moisture, ash and lipid) when they reach maturity. The second difference will be based on the path they take to reach maturity and how fast they reach it. Accord to Poklukar *et al. (*2020), modern pig breeds vary considerably from local breeds with regard to fat deposition, fat metabolism and variety of other traits. In addition, four pig breeds (Landrace, Yorkshire, Duroc and three-way crossbred LYD) have been studied by Choi *et al.* (2016) to investigate their chemical composition. It was found that there were significant differences in their chemical composition, with Duroc breed comprising of higher fat contents than all other breeds. Rokonuzzaman (2018) also reported significant variations in the moisture content in the wing of the three broiler strains, as well as the fat and protein composition of the drumstick.

It is well known that the chemical and physical composition of the body changes systematically as an animal grows (Emmans, 1995). In order to determine the changes in weight of various chemical components of the body at different growth stages, genotypes need to be described accurately. Prediction of carcass composition can be done using the interactions of carcass composition and the variations between males and females (Tumova *et al*., 2020). Despite the fact that effects of dietary protein reduction on several species of various genotypes and sexes have been investigated by the previous researchers (above mentioned), studies on responses of growing rabbit breeds and sexes in carcass composition are however scant or non-existent. Therefore, the aim of this study was to measure the responses of two growing rabbit breeds in carcass composition. The hypothesis tested in this study is that carcass composition is dependent on the level of dietary protein supplied.

4. 2 Materials and methods

4.2.1 Study Site

The study site has been described in detail in sections 3.2.1

4.2.2 Experimental design

Experimental designed was described in detail in section 3.2.2

4.2.3 Animal housing and management

Animal housing and management were described in section 3.2.3

4.2.4 Experimental diets

Experimental diets are described in section 3.2.4

4.2.5 Chemical composition of feeds

The chemical composition of the feed was also described in section 3.2.5

4.2.6 Slaughter procedure and carcass analysis

A total of eight (8) NZW rabbits (four males and four females) were sampled for physical and chemical analysis upon arrival to estimate the initial carcass composition of the NZW rabbits prior to being subjected to dietary treatments. At the end of the feeding trial, forty-eight rabbits (twenty-four NZW and twenty-four CAL rabbit breeds with equal numbers of males and females within each breed) were sampled for slaughter after eight hours of fasting to clear the gut contents. They were weighed prior to being transported to the abattoir for slaughter, reweighed prior to slaughter and the last weight measurements were taken after the pelt had been removed. These rabbits were transported in ventilated travelling cages in a closed vehicle suitably aerated to an approved certified local, Longleigh Poultry & Rabbit abattoir, ERF 59 Bishopstowe, Pietermaritzburg 325, where they were slaughtered by trained staff after being euthanized using electrical stunning, whereby electrical stunner electrodes were placed at the base of each ear. Manual evisceration was used. The rabbits were killed by cervical dislocation after being subjected to electrical stunning. After exsanguination, each rabbit was dissected appropriately so that the physical parts (whole carcass and pelts) of the body could be measured, whereby all the parts including the intestinal organs excluding pelts, were placed together and sealed together in a plastic bag, identified and placed in deep freeze (-20˚C) until mincing and chemical (moisture, protein, lipid, and ash contents) analysis was performed. Pelts and carcass were weighed and recorded.

4.2.7 Carcass composition parameters

4.2.7.1 Moisture content

Each rabbit carcass was cut into small pieces after being thawed followed by mincing the whole carcass three times using the mincing machine for homogeneity. The mincing machine was cleaned thoroughly with hot water and soap after mincing each carcass to prevent sample mixing. 150 g subsamples were weighed from each minced rabbit carcass and were freeze dried

on a Genevac SP Scientific freeze drying machine over 72 hours until a constant weight was reached to determine the moisture content. Weights of the freeze-dried samples were then recorded to determine moisture loss. The moisture content was then determined as the initial weight of the sample (150 g) minus the weight after freeze drying divided by the weight of the initial sample multiplied by 1000, with initial weight of the sample being W_1, weight after freeze drying (W_2) (AOAC 930.15). After freeze drying, the samples were further ground using a Retsch grinder to obtain a more homogenous mixture for further chemical analysis. The freeze-dried samples were then stored in desiccators for further analysis. The formula below was used to determine the moisture content.

$$Moisture = \frac{W_1 - W_2}{W_1} \times 1000$$

W_1 = Weight of the sample before freeze drying

W_2 = Weight of the sample after freeze drying

4.2.7.2 Ash determination

Ash content determination was performed according to (AOAC, 942.05). Crucibles with lids were dried in an oven at 90-105˚C overnight. They were then placed in the desiccator to cool. Crucible and lid were weighed, and their combined mass (W_1) was recorded. A well-mixed dry sample of approximately 1g was weighed and recorded. This was a total mass of the crucible, lid, and sample (W_2). The weighed crucible with the sample and lid was then placed in the furnace at 550°C, overnight. The furnace was however allowed to cool to less than 200°C before removing the tray containing these crucibles. The crucibles were then placed in the desiccator to cool. After they have cooled, their mass was weighed and recorded (W_3). Ash % was then determined as:

$$Ash\% = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where:

W_1 = Crucible and lid mass

W_2 = Crucible with lid and sample mass

W_3 = Ashed crucible with lid and sample mass

4.2.7.3 Fat

The extraction of fat was done using the Soxhlet procedure according to AOAC (920.39). Buchi fat beakers were placed in the oven to dry overnight. A thimble was placed on the balance

and the balance was tared to zero. A dried ground sample of approximately 5g was weighed into the thimble and its mass was recorded as W_1. It was then placed in the oven to dry for 1 hour since it is important that fat extraction is carried out on a dry sample. The Buchi fat beaker was weighed with extraction stones and its mass was recorded as W_2. Each thimble was then paired to a flask for numbering purposes. The thimbles were then sealed with cotton wool and petroleum was poured into the beakers approximately ¾ (three quarters) full. Six thimbles were then placed, one in each Soxhlet extractor. The beakers were placed onto the heating spaces. The glass sections were closed and sealed together. The cooling water supply was then turned on and leaks were checked. The extraction process was allowed to take place for 4 hours. The flow of refluxed fat-free solvent was redirected to collect in container at the back of the machine. The whole system was opened, and thimbles and beakers were removed when the flasks all appeared to be free of solvent. These thimbles and beakers were then left overnight on the bench to allow the remaining solvent to evaporate. The beakers were dried in the oven at 90˚C for an hour. They were then allowed to cool in the desiccator. Their masses were weighed and recorded (W_3). Crude fat (%) was then determined as:

$$Crude\ Fat\ \% = \frac{W_3 - W_2}{W_1} \times 100$$

Where:

W_1 = Mass of a sample (g)

W_2 = Mass of the Buchi fat beaker (g)

W_3 = Mass of the Buchi fat beaker with extracted residue (g)

4.2.7.4 Protein

Protein analysis was carried out using the Dumas combustion method which quantifies nitrogen of the sample. A 0.2 g sample undergoes complete combustion at high temperatures (900-950℃) in the presence of oxygen (99.9%). Water, carbon dioxide, nitrogen dioxide and nitrogen oxides, are the combustion products. A sequence of thermoelectric coolers and chemical sorbents remove water vapor, oxygen, and carbon dioxide from the resultant gas steam. The combustion products are then collected and allowed to equilibrate. When nitrogen passes through a column of heated copper, it is converted to N2. The resultant N2 is then measured using a thermal conductivity detector. Protein is then determined from nitrogen content using a known conversion factor of 6.25 (AOAC 992.15).

4.2.8 Statistical analysis

The treatment means were determined by analysis of variance using ANOVA in GenStat (20th edition, VSN International, 2022). Exponential regression and simple linear regression with groups, groups being breed and sex were used. Significance was declared at $P < 0.05$.

Linear model: $y = a \pm bx$

Where:

Y = Variate being regressed

a = Constant term

b = Regression coefficient

x = dietary protein level

Exponential Model: y = A + B* (R**X)

Where:

A + B is the y-intercept

R is the exponential base

X is the level of the balanced protein

4.3 Results

4.3.1 Response in carcass composition to balanced dietary protein

Overall mean values of moisture, ash, fat, and protein contents of the NZW and CAL female and male rabbits slaughtered after an eight-week feeding experiment, are shown in Table 4.1. Mean carcass and pelt weights (g) are demonstrated in Table 4.2. Exponential regression and simple linear regression with groups were used to measure the responses; groups were breed and sex. Regression coefficient tables for variables of interest are given from Tables 4.3 to 4.14. As shown in exponential and linear regression tables, results on moisture, lipid, ash and protein content did not have a significant association with balanced dietary protein ($P > 0.05$). The lowest and second lowest protein contents resulted in higher lipid contents (178 and 183g/kg) in female and male NZW rabbits, respectively. CAL rabbits on the highest level of dietary protein had highest body lipid contents. Highest body protein content was found on rabbits on the highest dietary protein level in both sexes of the NZW and CAL males. The CAL breed had higher ash, fat and protein contents and subsequently lower moisture content than the NZW rabbits. Significant sex differences in moisture, lipid and protein response to dietary protein content were found on the intercepts of the sexes ($P < 0.05$). Dietary protein influenced the responses in carcass and pelt weights of the two rabbit breeds ($P < 0.05$). Higher carcass

weights were found on the CAL breed with CAL females having the highest carcass weight whereas highest pelt weights were observed on the NZW breed. Significant interactions were observed between the females of the two breeds in carcass and pelt weights. Three-way significant interactions were evident in carcass and pelt weights of the females of the two breeds.

Table 4. 1 Mean moisture, ash, lipid and Protein content in the carcass of NZW (initial and final) and CAL female and male rabbits fed a range of dietary protein levels for a period of 56 d.

	Moisture (g/kg)				Ash (g/kg)				Fat (g/kg)				Protein (g/kg)			
	NZW		CAL		NZW		CAL		NZW		CAL		NZW		CAL	
Protein g/kg	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Initial (NZW)	671	666			55	49			102	91			172	194		
126	637	603	643	650	31	28	34	29	152	183	118	130	180	186	205	191
143	620	660	593	620	57	39	65	35	178	134	168	152	145	167	173	193
161	657	627	577	613	33	27	59	44	107	167	183	159	203	179	181	184
178	663	633	610	617	42	45	31	69	101	132	176	148	194	190	183	166
196	645	607	630	590	49	38	28	51	137	175	147	172	169	180	195	186
213	643	627	577	627	56	47	75	34	94	129	153	138	207	197	195	201
MEAN	644	626	605	618	45	37	49	44	128	153	158	150	183	183	189	187
		SEM[1]	P-value			SEM	P-value			SEM	P-value			SEM	P-value	
Protein		11.8	0.9			3.1	0.2			4.7	0.03			13.9	0.7	
Breed		6.8	0.03			1.8	0.8			2.7	0.6			8.01	0.3	
Sex		6.8	0.8			1.8	0.1			2.7	0.1			8.01	0.8	

SEM[1], Standard error of mean

Table 4. 2 Mean carcass and pelt weights of NZW (initial and final) and CAL male and female rabbits fed a range of dietary protein levels over 56 d period.

	Carcass weight (g)				Pelt weight (g)			
	NZW		CAL		NZW		CAL	
Protein g/kg	F	M	F	M	F	M	F	M
Initial (NZW)	500	625			150	175		
126	1750	1650	1550	1500	475	500	375	375
143	1675	1800	1750	1600	475	500	450	425
161	1700	1850	1875	1625	525	500	475	450
178	1600	1725	1975	1825	450	475	550	475
196	1525	1925	2025	1700	450	550	550	450
213	1650	1900	2075	1825	450	525	550	450
MEAN	1650	1808	1875	1679	471	508	492	438

	SEM[1]	P value	SEM	P value
Protein	60.1	0.112	23.25	0.353
Breed	34.7	0.339	13.42	0.166
Sex	34.7	0.706	13.42	0.914

SEM[1], Standard error of mean

Table 4. 3 Exponential regression coefficients describing carcass moisture content (g/kg) of female and male NZW and CAL rabbits over 56 d period.

Coefficient	NZW		CAL	
	F	M	F	M
A	646	631	597	613
B	-4.655E+108	-1.363E+109	2.294E+109	1.829E+109
R	0			
	R^2 = 0.69			

Table 4. 4 Linear regression coefficients of the moisture content (g/kg) of male and female NZW and CAL rabbits subjected to dietary protein levels over 56d period.

Parameter	Estimate	s.e	t (40)	t pr.
Constant	657.3	54.0	12.16	<0.001
Protein	-0.308	0.314	-0.98	0.332
Br x Sex CM	18.8	76.4	0.25	0.807
Br x Sex NF	-46.3	76.4	-0.61	0.548
Br x Sex NM	-20.3	76.4	-0.27	0.792
PROTEIN.Br x Sex CM	-0.026	0.444	-0.06	0.954
PROTEIN.Br xSex NF	0.506	0.444	1.14	0.261
PROTEIN.Br x Sex NM	0.244	0.444	0.55	0.585
R^2 = 0.77				

Factor Reference level

Br x Sex CF

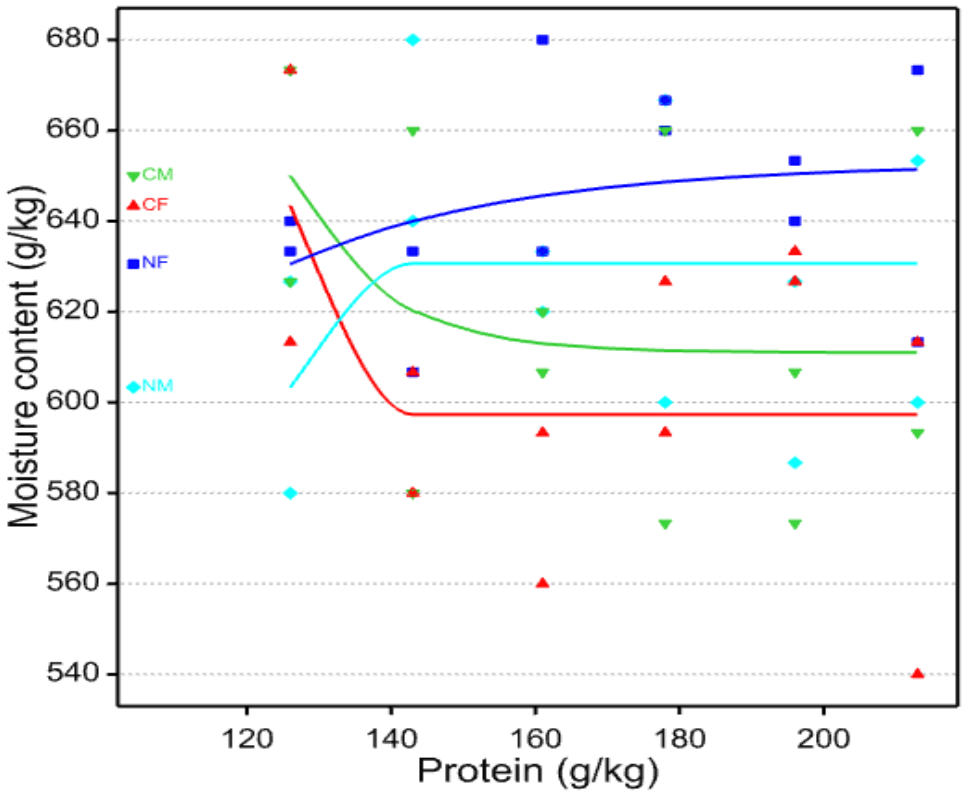

Figure 4.1 Fitted and observed exponential relationship between dietary protein content and carcass moisture content of CAL and NZW female and male rabbits.

Table 4. 5 Exponential regression coefficients describing the carcass ash content (g/kg) of female and male NZW and CAL rabbits over 56 d period.

Coefficient	NZW		CAL	
	F	M	F	M
A	10.9	8.8	10.7	9.8
B	-2.700E+126	-2.581E+126	-2.069E+126	-2.052E+126
R	0.1002			
	R^2 = 0.85			

Table 4. 6 Linear regression coefficients of the ash content (g/kg) of male and female NZW and CAL rabbits subjected to dietary protein levels over 56 d period.

Parameter	Estimate	s.e	t (40)	t pr.
Constant	28.9	15.5	1.86	0.070
Protein	-0.0027	0.0902	-0.03	0.976
Br x Sex CM	-8.0	21.9	-0.37	0.717
Br x Sex NF	-25.9	21.9	-1.18	0.244
Br x Sex NM	-23.2	21.9	-1.06	0.297
PROTEIN.Br x Sex CM	0.041	0.128	0.33	0.747
PROTEIN.Br x Sex NF	0.176	0.128	1.38	0.176
PROTEIN.Br x Sex NM	0.118	0.128	0.92	0.361
R^2 = 0.81				

Factor Reference level

Br x Sex CF

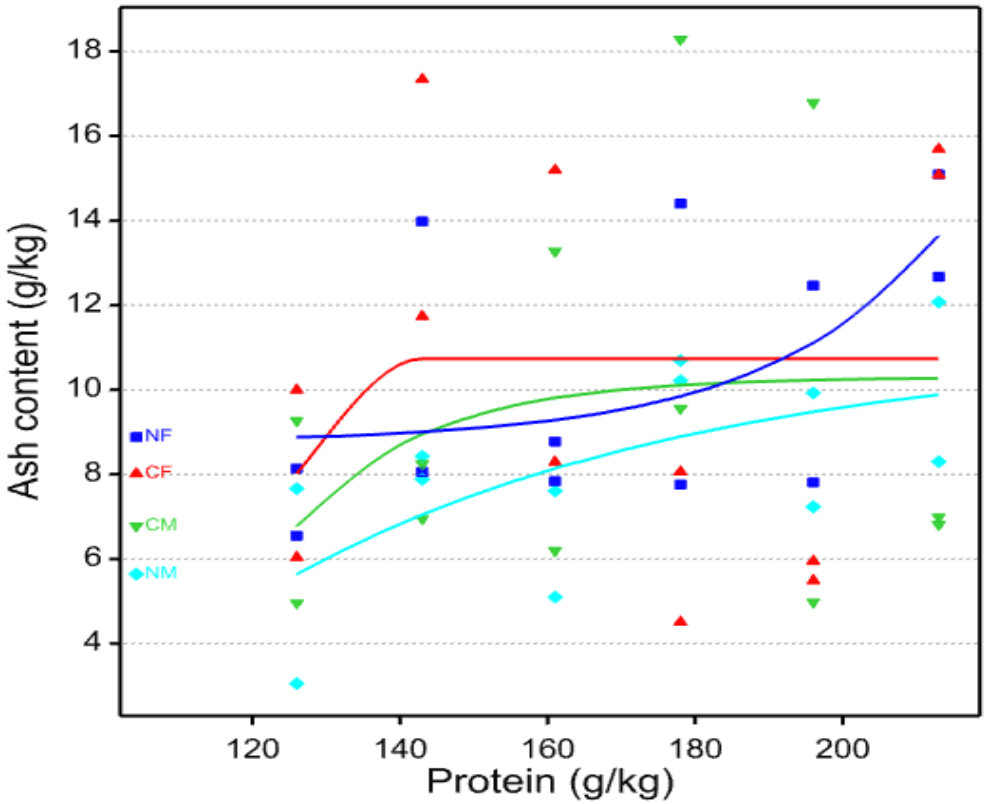

Figure 4.2 Fitted and observed exponential relationship between dietary protein content and carcass ash content of CAL and NZW female and male rabbits.

Table 4.7 Exponential regression coefficients describing the carcass fat content (g/kg) of female and male NZW and CAL rabbits over 56 d period.

Coefficient	NZW		CAL	
	F	M	F	M
A	29	36	38	35
B	170982	76950	-92710	-1421
R	0.9270			
	R^2 = 0.75			

Table 4.8 Linear regression coefficients of the Fat content (g/kg) of male and female NZW and CAL rabbits subjected to dietary protein levels over 56 d period.

Parameter	Estimate	s.e	t (40)	t pr.
Constant	103.4	22.5	4.59	<.001
Protein	-0.053	0.131	-0.41	0.685
Br x Sex CM	17	31.8	0.53	0.597
Br x Sex NF	52.7	31.8	1.66	0.106
Br x Sex NM	31	31.8	0.97	0.336
PROTEIN.Br x Sex CM	-0.091	0.185	-0.49	0.627
PROTEIN.Br x Sex NF	-0.324	0.185	-1.75	0.087
PROTEIN.Br x Sex NM	-0.132	0.185	-0.72	0.478
R^2 = 0.71				

Factor Reference level

Br x Sex CF

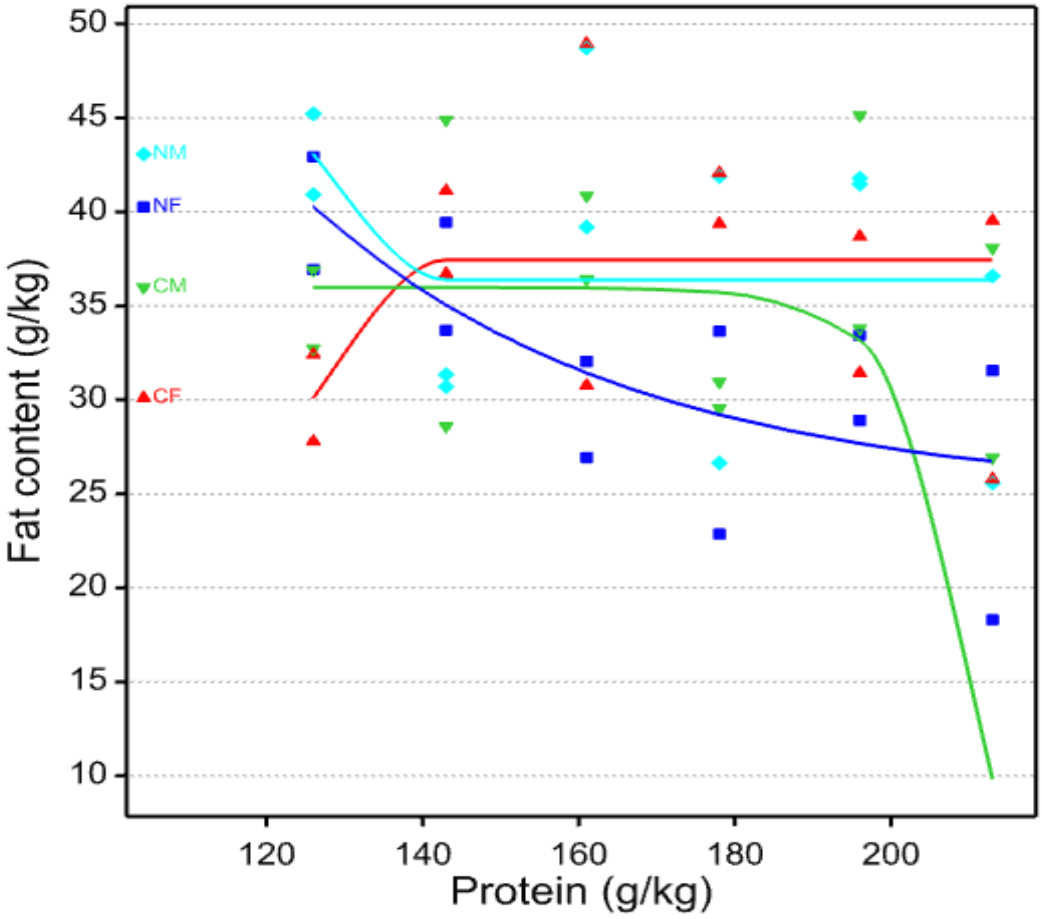

Figure 4.3 Fitted and observed exponential relationship between dietary protein content and carcass fat content of CAL and NZW female and male rabbits.

Table 4. 9 Exponential regression coefficients describing the carcass protein content (g/kg) of male and female NZW and CAL rabbits over 56 d period.

Coefficient	NZW		CAL	
	F	M	F	M
A	48	45	41	41
B	-12373	-9764	23143	24638
R	0.9400			
	R^2 = 0.92			

Table 4.10 Linear regression coefficients of the protein content (g/kg) of male and female NZW and CAL rabbits subjected to dietary protein levels over 56 d period.

Parameter	Estimate	s.e	t (40)	t pr.
Constant	158	62.3	2.54	0.015
Protein	-0.253	0.362	-0.70	0.489
Br x Sex CM	11.6	88.1	0.13	0.896
Br x Sex NF	-81	88.1	-0.92	0.363
Br x Sex NM	-56.4	88.1	-0.64	0.526
PROTEIN.Br x Sex CM	-0.034	0.152	-0.07	0.947
PROTEIN.Br x Sex NF	0.01	0.152	1.17	0.248
PROTEIN.Br x Sex NM	0.381	0.152	0.74	0.461
R^2 = 0.91				

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Br x Sex	CF

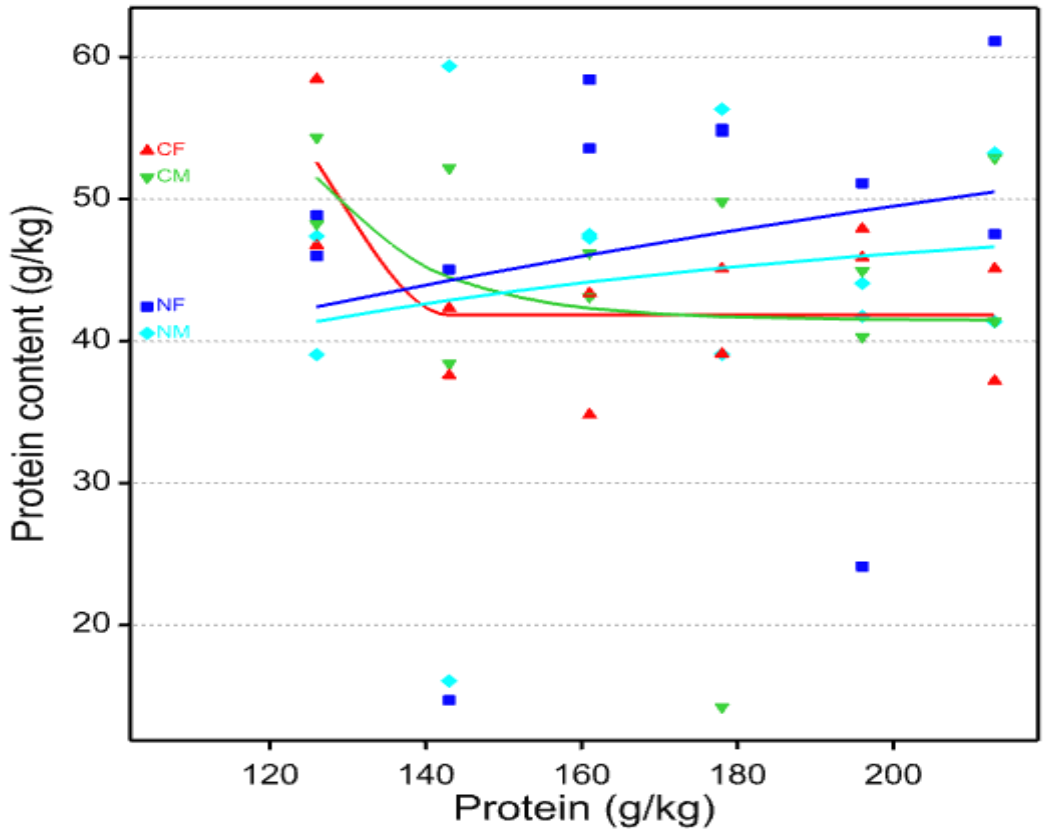

Figure 4.4 Fitted and observed exponential relationship between dietary protein content and carcass protein content of CAL and NZW female and male rabbits.

Table 4.11 Exponential regression coefficients describing the carcass weight (g) of male and female NZW and CAL rabbits over 56 d period.

Coefficient	NZW		CAL	
	F	M	F	M
A	1.56	1.93	2.17	1.85
B	2.89	-3.94	-9.41	-5.53
R		0.9786		
		R^2 = 0.44		

Table 4. 12 Linear regression coefficients of the carcass weight (g) of male and female NZW and CAL rabbits subjected to dietary protein levels over 56 d period.

Parameter	Estimate	s.e	t (40)	t pr.
Constant	891	241	3.69	<.001
Protein	5.81	1.40	4.14	<.001
Br x Sex CM	201	341	0.59	0.559
Br x Sex NF	1051	341	3.08	0.004
Br x Sex NM	501	341	1.47	0.150
PROTEIN.Br x Sex CM	-2.34	1.98	-1.18	0.245
PROTEIN.Br x Sex NF	-7.53	1.98	-3.79	<.001
PROTEIN.Br x Sex NM	-3.35	1.98	-1.69	0.099
R^2 = 0.46				

Factor Reference level

Br x Sex CF

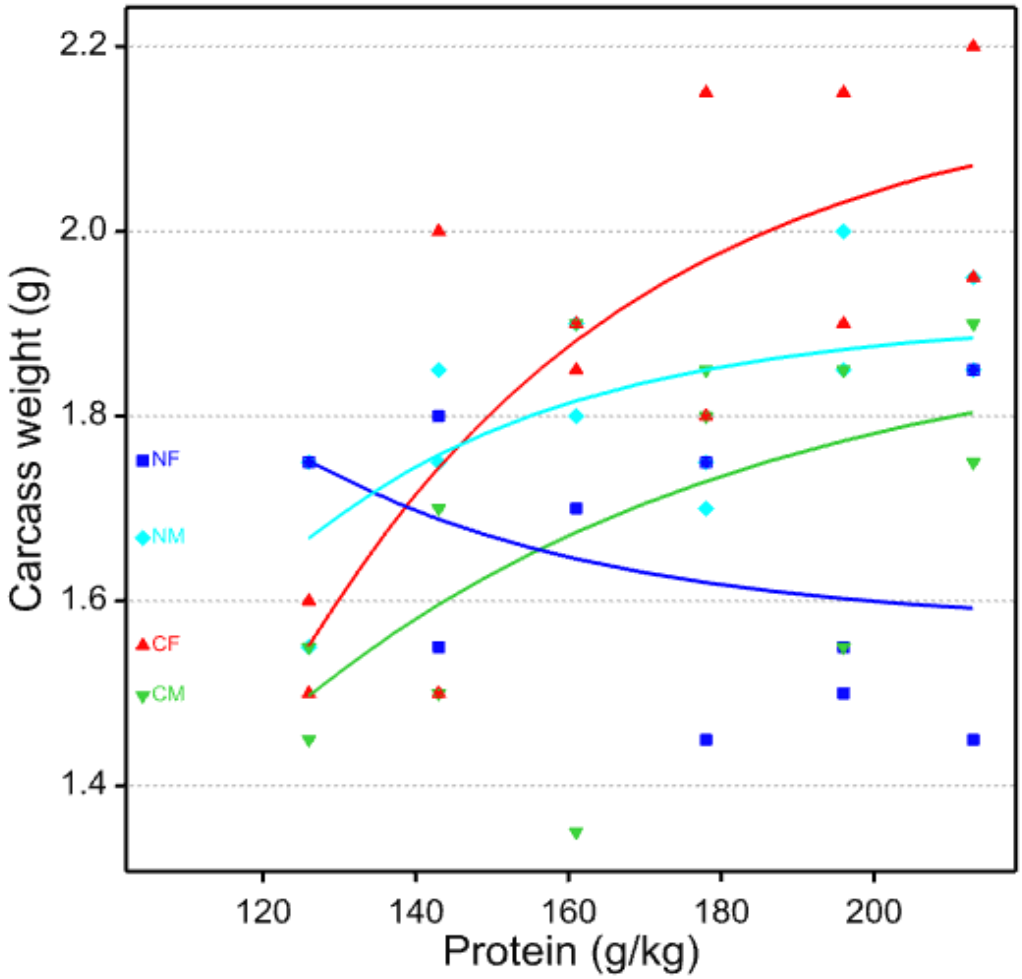

Figure 4.5 Fitted and observed exponential relationship between dietary protein content and carcass weights of CAL and NZW female and male rabbits.

Table 4.13 Exponential regression coefficients describing the pelt weight (g) of female and male NZW and CAL rabbits over 56 d period.

Coefficient	NZW		CAL	
	F	M	F	M
A	463	517	542	476
B	2320	-2362	-16410	-9648
R	0.9643			
	R^2 = 0.61			

Table 4.14 Linear regression coefficients of the pelt weights (g) of male and female NZW and CAL rabbits subjected to dietary protein levels over 56 d period.

Parameter	Estimate	s.e	t (40)	t pr.
Constant	206.1	96.6	2.13	0.039
Protein	1.636	0.562	2.91	0.006
Br x Sex CM	70	137	0.51	0.614
Br x Sex NF	341	137	2.49	0.017
Br x Sex NM	232	137	1.70	0.097
PROTEIN.Br x Sex CM	-0.656	0.794	-0.83	0.414
PROTEIN.Br x Sex NF	-2.084	0.794	-2.62	0.012
PROTEIN.Br x Sex NM	-1.223	0.794	-1.54	0.131
R^2 = 0.66				

Factor	Reference level
Br x Sex	CF

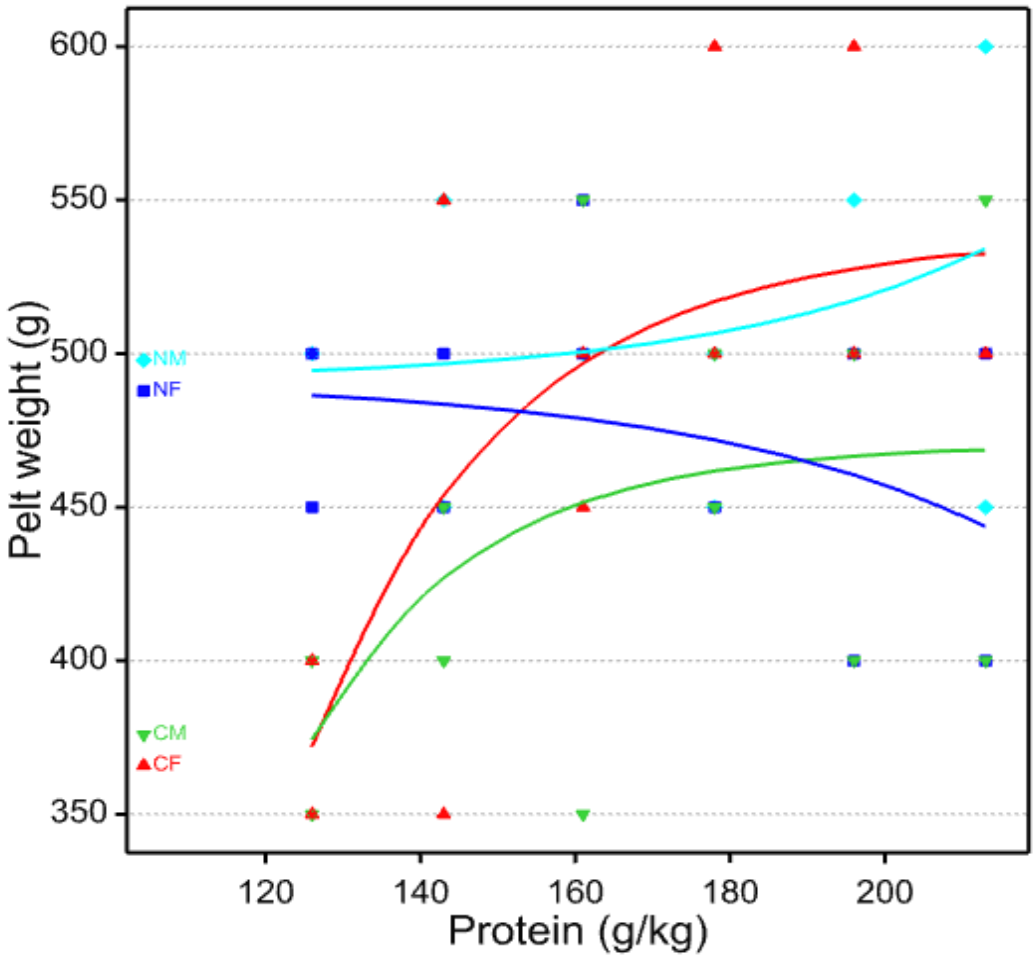

Figure 4.6 Fitted and observed exponential relationship between dietary protein content and pelt weights of CAL and NZW female and male rabbits.

4.4 Discussion

This study was designed to measure the response of growing rabbits in carcass composition to balanced dietary protein. The findings of this study showed that moisture, ash, lipid, and protein contents were all not influenced by the dietary protein content ($P > 0.05$), which is consistent with the previous research that also did not observe any significant influence of dietary protein on whole body composition of broilers (Kamran *et al*., 2008; Quentin *et al*.,2003) and pigs (Sirtori *et al.*,2014) following a reduction in dietary crude protein levels. No significant differences and interactions were detected between the two breeds and sexes ($P > 0.05$), nor there were any significant interactions observed between protein level, breed, and sex. This implies that the two rabbits breeds and sexes used in this study responded similarly in carcass composition. This similar response may be due to less variations resulting from small numbers of rabbits sampled from each dietary treatment (n=8). Even though these breeds showed similar responses but there were visible differences observed. Moisture content was numerically higher in the NZW breed in both sexes than CAL breed, 644 g/kg vs 605 and 626 vs 619 g/kg in females and males, respectively. However, the initial moisture content of the NZW breed of both sexes was higher at weaning (671 and 666 for females and males) than at 14 weeks (643 and 627 for females and males) of age when they were slaughtered. A decrease from 671 to 644 (27% decrease) was observed in the NZW females whereas a decrease from 666 to 626 (40% decrease) was observed in males.

These results are in accordance with the results of Vargas *et al*. (2020) who observed a decrease from 777 to 645 g/kg in the water content of the feather-free body weight of broilers of two strains (Robb and Cobb). However, Emmans (1989) also stated that moisture content decreases as an animal grows because wetter tissues are becoming replaced by the dry ones during growth and also because wet tissues become dry as an animal grows. The CAL breed was unfortunately not sampled for the initial carcass composition at the beginning of the trial as the number of rabbits from this breed were low and not sufficient for the sampling for the initial carcass composition. However, it is believed that they would have also shown similar responses of decreasing moisture content during growth. According to the study of Hermes *et a*l. (1999), it was discovered that Californian rabbits slaughtered at 16 weeks had lower moisture contents than the ones slaughtered at 12 weeks. Bieniek *et al*. (1994) also found a decreased moisture content in 140-day rabbits than in 60-day rabbits and the findings of these researchers however

support the assumptions about the CAL rabbits which would have also shown decreased moisture content due to age effect.

The CAL breed had higher ash contents than the NZW with females comprising of the highest ash content. However, highest ash contents were found on the highest dietary protein levels in the NZW breed and CAL females whereas the high ash contents were observed on the 178 and 196 g/kg in the CAL males. Protein to ash ratios were found to be 4.07, 4.9, 3.9 and 4.3. Decreases from 55 to 45 g/kg (10%) and 49 to 37 g/kg (12%) in the ash contents of weaned (6 weeks) and 14-week-old female and male NZW rabbits respectively were observed.

New Zealand White male fat content increased from an original estimate of 91 to 153 g/kg (62% increase), whereas female fat (lipid) content increased from 102 to 128 g/kg (26% increase) and these differences were expected due to the known fact that the carcass composition of animals changes as an animal grow. The fat gain may be related to body weight differences that these rabbits had at slaughter as they were slaughtered at weaning and 14 weeks (Clark *et al*., 2019).

As expected according to Gous *et al*. (2012), NZW rabbits (both males and females) showed highest lipid contents on the lowest (126 g/kg) and second lowest (143) dietary protein levels than on the highest protein level. The amount of fat in the carcass increases when a diet lower in protein than recommended is consumed. Dietary protein content ranges to meet protein requirements of growing rabbits were recommended by Marín García (2019), and these values were 158-178 g/kg. However, the high lipid contents were observed on the dietary protein level (126 g/kg) lower than these recommended values, hence the observed increase in the lipid content.

As stated above, both males and females of the NZW breed had the highest fat contents on the lowest and second lowest dietary protein levels. This may be also because when animals are provided with insufficient dietary protein content, excess energy may be diverted to fat deposition. These findings are however consistent with those of several authors (Summers and Leeson, 1985; Parr and Summers, 1991; Deschepper and de Groote, 1995) who observed increased body fat content in birds fed low-protein diets.

Concerning the CAL rabbits, CAL females and males had the highest fat content on the 161 g/kg dietary protein content and second highest protein content (196 g/kg), respectively. This response is however insignificantly different from the response observed in the NZW rabbits.

According to Siebrits *et al.* (1986), breed specific variations are evident in terms of protein deposition and carcass composition.

These observations contrast the findings of various authors (Gloaguen *et al*., 2014; Ruusunen *et al*., 2007; Morazán *et al*., 2015) who concluded that low protein diets result in increased fat carcass contents. However, the differences in fat contents of the two breeds were expected from the significant varying responses in the feed intake of these breeds. The low lipid levels of the CAL rabbits observed on the highest protein level demonstrate a response to the increased protein intake. These observations are in agreement with the results of Summers and Leeson (1984), who concluded that an increase in dietary protein level results in the reduction of the fat content of the edible portion.

The NZW males had higher mean fat content than the CAL males. On the other hand, CAL females had the highest overall mean fat content and this could be associated with varying genotype in comparison with the NZW rabbits. Concerning the CAL breed this difference may be due different nutritional needs and metabolism (Tůmová and Teimouri, 2010), relatively higher hormonal influences in females and higher ability of fat accretion of these different sexes.

As for protein body content, the estimated initial protein content was higher in NZW males than in females and this may be due to the implication that males have inherent higher body weights than females which may in turn influence protein deposition.

In comparison to the NZW breeds, CAL rabbits had higher body protein content. Low body protein contents of the NZW rabbits may be associated with low feed intakes. Highest body protein contents were observed on the highest dietary protein level on both sexes of the NZW breed and the CAL male rabbits. CAL females had the highest overall protein content. This means that these animals were able to efficiently convert dietary protein content in feed into body protein although this was not statistically significant. Both sexes of the CAL breed demonstrated higher protein body content than their counterparts. This may be due to the higher ability of protein accretion of this breed. It is however believed that genetic variations can greatly influence the rate of protein deposition. Maximum protein deposition in the NZW breed was in the rabbits receiving the highest dietary protein content (213g/kg) in both females and males. This highest body protein contents found on the highest dietary protein level was however expected since high dietary protein content results in increased carcass protein content (Bogosavljević-Bošković *et al*.,2010). The highest body protein content in the CAL female and

male rabbits was observed on the lowest and highest dietary protein level, respectively. These variations may be based on different metabolism of the different sexes.

Carcass and pelt weight demonstrated a significant linear response to balanced dietary protein ($P<0.05$). Significant interactions (Protein x breed x sex) and (breed x sex) were observed in carcass and pelt weights of females of the two breeds. This shows that the females of these breeds responded differently to balanced dietary protein. New Zealand White females increased carcass and pelt weights in response to decreased dietary protein, while the CAL females decreased their carcass and pelt weights with decreasing protein content. The maximum carcass weights of NZW females were therefore recorded on the low dietary protein levels (126g/kg and 143g/kg), whereas the highest carcass weights of CAL females were detected on the high dietary protein levels (178g/kg, 196g/kg, and 213g/kg). The results of improved carcass weights of the NZW females in response to decreased dietary protein content agrees with the findings of Nørgaard *et al*. (2014), who observed increased pig carcass weights in response to decreased crude protein content.

4.5 Conclusions

It can be concluded that the two breeds used in this study have different lipid and protein growth potential even though these body components did not differ significantly between these breeds. Sexes of the same and different breeds differ in their responses due to their different nutritional requirements and metabolic functions.

Considerable variation within treatments in all responses measured in this study meant that the responses to dietary protein could not be accurately described. Solutions to this challenge would be to use more rabbits per treatment, to sample more rabbits per treatment, and to check on the accuracy of the laboratory analyses. Widening the range of dietary protein levels may result in a greater difference in the response of the rabbits to protein, thereby describing this response more accurately.

Chapter 5

5.1 General discussion

The broad objective of the current study was to measure the responses in growth performance and carcass composition of growing rabbits to balanced dietary protein. The hypothesis tested was that this measured response depends on the level of dietary protein supplied. As this response was also expected to be influenced by genotype and sex, two breeds and sexes of rabbits were then used in this regard. The findings of this study revealed that the rabbits of the two breeds and sexes responded differently in growth performance indicating that the optimum protein levels to include in diets of these animals should differ in an attempt to achieve maximum production. The need for further research is however emphasized in this study in order to corroborate the findings of this study and estimate the precise ideal dietary protein level to include in growing rabbit diets for maximum performance and profitability.

It can also be theorized that performance decline would occur when the dietary protein levels in rabbit diets fall below those of the whole-body composition if rabbit whole body protein composition can be used, at least as a first estimation, towards the determination of the ideal dietary protein level required for rabbit growth and maintenance. Use of protein body composition to estimate maintenance requirements is mainly recommended as the lipid content of the body has been shown to vary considerably and it is hugely affected by the diet supplied to the growing animal.

Determination of protein responses of growing rabbits have never been evaluated. In order to achieve this, it is necessary to understand the changes that occur in protein body composition during growth. In this study, NZW females increased their protein body composition from 172 g/kg when they were six weeks old to 183 g/kg at fourteen weeks. Whereas on the other hand, the NZW male rabbits decreased their protein body composition from 197 g/kg which was determined at weaning age to 183 g/kg at 14 weeks.

Inadequate and excess dietary protein levels in diets of animals have been shown to result in decreased growth and withdrawal of protein in body tissues. Therefore, dietary protein levels must be supplied in adequate amounts that will meet the nutritional needs of animals as this is crucial for maximum productivity. It is important to note that this is a challenge to overcome as the nutritional needs of animals are not constant and are varying within animals of the same flock. Protein requirements of growing rabbits have also been shown to vary according to physiological state of an individual animal.

Genotype is another factor that influences the protein needs of growing animals and this was evident based on our findings. On closer observation of the results of this study, it is obvious that the two breeds used in this study have different nutritional needs. Based on the intersecting lines of the feed intake graph, it was clearly evident that the other breed was attempting to meet its protein requirements by increasing its feed intake as a response to the decreased dietary protein levels. As both sexes of the CAL breed decreased their feed intake in response to protein reduction, neither of these sexes conformed to the theory proposed by Emmans (1981 and 1989), which states that a growing animal will consume more of the provided feed if it is limited in nutrient supply to meet its nutrient needs. As a consequence, the CAL breed had a lower growth rate compared to the NZW rabbits.

Predicting feed intake is also a common challenge in livestock production. This is mainly because there are many factors that influence feed intake of animals. Furthermore, it is difficult to examine the interactions of these factors simultaneously. The protein content in the rabbit carcass could be used to estimate the protein requirements of growing rabbits. Comparing the findings of this study with other studies on protein response of growing rabbits in carcass whole body compositions poses a challenge because there is a lack of available data on protein response. Therefore, future research could focus on repeating this experiment on growing rabbits with the same breeds as the ones that were used in this study and if possible with more rabbit breeds. Therefore, future research may also focus on repeating this experiment with growing rabbits using the same breeds that were used in the current study and, if possible, with more breeds of rabbit for an accurate estimation of the optimum protein level to include in rabbit diets and for proper estimation of maintenance requirements of growing rabbits.

5.2 Future research

Future research could repeat this experiment to corroborate these findings and /or focus on modeling various breeds of rabbits. Higher sample numbers for carcass analysis would be an advantage given the high variation within each treatment. Also, widening the range of dietary protein levels may result in a greater difference in the response of the rabbits to protein, thereby describing this response more accurately.

5.3 Study limitations

There was a limited number of Californian rabbits among the two breeds that were used. It would have been ideal to use more breeds, with more rabbits per breed, as the population size is also considered to influence the response of growing animals in growth performance and carcass composition.

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