Determination of Optimal Levels of Energy, Protein, and Fiber in the Diets of New Zealand White Growing Rabbits Based on Nutrient-Response Models

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Abstract

A study was conducted to estimate the optimal levels of energy, protein, and fiber in the diets of New Zealand White (NZW) growing rabbits fed on fresh green forage available in North Vietnam. Mathematical modelling of nutrient-response curves was applied for the study. A total of 125 male growing rabbits at 6 weeks of age were randomly divided into 25 groups of 5 each to be fed on diets with an array of different levels of metabolizable energy (ME), crude protein (CP), and acid detergent fiber (ADF) by means of varying the ratio between fiber rich grasses, *viz.* elephant grass (*Pennisetum purpureum*), Setaria grass (*Setaria sphacelata*), or Para grass (*Brachiaria mutica*), and protein rich forages, *viz.* water spinach (*Ipomoea aquatica*) vines, sweet potato (*Ipomoea batatas*) vines, or *Trichantera* leaves (*Trichanthera gigantea*), in the basal diets. The average daily gain (ADG) and feed conversion ratio (FCR) were used as key responses of the rabbits to varied levels of ME, CP, and ADF in the diets. The results showed that the levels of ME, CP, and ADF in the diets strongly affected the ADG and FCR of the rabbits following curvilinear patterns with the highest ADG and lowest FCR when the diet contained 2106-2162 Kcal ME kg^{-1} DM with 16.5-16.8% CP and 21.9-22.4% ADF. Therefore, these levels of nutrients were considered to be optimal in the diets of NZW growing rabbits fed on local forages.

Keywords

Nutrient-Response Model, Rabbits, Energy, Protein, Fiber

Introduction

In developed countries, rabbits are normally fed high starch-based diets (Xiccato *et al.*, 2011). Starch is the main source of energy for feeding rabbits, however, high-starch low-fiber diets

have been considered responsible for digestive troubles for a long time (Blas & Gidenne, 2010; De Blas & Mateos, 2010). In addition, a high dietary protein level, besides affecting growth performance and nitrogen excretion of rabbits (Xiccato & Trocino, 2010), may impair caecal fermentation and alter gut microflora composition, and thus is considered to be among the causes of increased mortality in rabbits (De Blas *et al.*, 1999; Carabaño *et al.*, 2009). Otherwise, the substitution of starch with soluble fiber has been shown to often reduce mortality (Gidenne & Perez, 2000; Soler *et al.*, 2004; Xiccato *et al.*, 2008). Moreover, the starch-based and high protein diets used in developed countries are mainly due to high labor costs; but these diets may remove the significant advantage of rabbits as herbivores (Leng, 2008). Therefore, the development of efficient rabbit diets should be based on locally available forages of high nutritive value in developing countries.

In fact, feeding exotic rabbits on locally available forages has recently been very popular in Vietnam. Several studies in the country have revealed that feeding rabbits with local feeds can maintain their high growth rate and efficient feed conversion (Luyen & Preston, 2012). Tam *et al.* (2009) and Huyen *et al.* (2013a, 2013b) found that the average daily gain (ADG) was improved and the feed conversion ratio (FCR) reduced when New Zealand White (NZW) rabbits were fed a protein-rich forage-based diet (water spinach or sweet potato vines) supplemented with energy and/or fiber-rich feeds (paddy rice, broken rice, or broken rice with added rice husks). Similarly, Hang *et al.* (2013) found linear increases in the growth rate of NZW growing rabbits when forage-based diets were supplemented with paddy rice up to 20g/head/day. However, these studies only focused on the levels of replacement and/or supplementation of different local feeds but did not provide any information on the optimal levels of nutrients in the diet. It is clear that knowledge of nutrient requirements or nutrient levels in a diet based on local forages of rabbits in general, and NZW rabbits in particular, is still limited. Therefore, there is a high need for research to determine the optimal levels of nutrients in the diets of exotic rabbits fed on forages available in the country. The present paper reports an experiment to estimate optimal levels of metabolizable energy (ME), crude protein (CP), and acid detergent fiber (ADF) in the diets of NZW growing rabbits based on feed resources available in North Vietnam using the method of mathematically modelling nutrient-response curves.

Materials and Methods

Animals and diets

A total of 125 male NZW growing rabbits at 6 weeks of age were randomly allocated into 25 groups of 5 each to be fed on different diets based on fiber-rich grasses (Elephant grass, Setaria grass, or Para grass) mixed with protein-rich forages (water spinach vine, sweet potato vine, or Trichantera leaves) (**Table 1**) at varied proportions (0, 25, 50, 75, and 100%) to make a large array of ME, CP, and ADF levels (**Table 2**). In addition to the green fodder, the rabbits were supplemented with paddy rice at 2% of their body weight (BW). The levels of ME, CP, and ADF in the diets varied in large ranges (**Table 3**) that covered all the spectra of respective values of the nutrients in the diet recommended for rabbits by NRC (1977), Lebas (1980), and other studies (Tao & Li, 2006; De Blas & Wiseman, 2010; Halls, 2010; Osho *et al.*, 2013).

Feeding management

The experiment was carried out at the experimental animal farm of Vietnam National University of Agriculture for 8 weeks after a period of 7 days of adaptation. Before the experiment began, the rabbits were vaccinated against hemorrhagic disease using the VHD vaccine and drenched against coccidiosis using an ANTICOC product. At each time of feeding, feeds were chopped and mixed according to the designed proportions. Rabbits were fed green fodder three times a day at 8:00, 14:00, and 20:00. Paddy rice was fed at 11:00 am every day. Water was made available at all times.

Table 1. Chemical composition and metabolizable energy values of the experimental feeds

Feed	DM (%)	CP (% DM)	NDF (% DM)	ADF (% DM)	CF (% DM)	EE (% DM)	Ash (% DM)	ME (Kcal kg^{-1} DM)
Elephant grass	14.43	14.44	62.36	33.14	28.89	0.62	13.42	1827
Setaria grass	13.67	15.29	60.38	33.21	28.87	2.86	11.03	1990
Para grass	17.17	11.26	76.39	35.35	31.40	0.59	11.42	1875
Water spinach vine	11.82	26.79	30.07	19.72	20.42	1.32	11.95	2445
Sweet potato vine	11.00	23.06	37.76	22.45	19.78	2.55	11.24	2398
Trichantera leaves	17.45	18.82	45.76	25.46	24.41	2.27	14.27	2058
Paddy rice	88.01	6.94	32.19	16.60	13.86	0.29	8.10	2819

Table 2. Design of the experimental diets

PAR100 & TRI0	ELE100 & WAS0	SET100 & SWP0	WAS & THOC0	SWP & RICE0
PAR75 & TRI25	ELE75 & WAS25	SET75 & SWP25	WAS & THOC1	SWP & RICE1
PAR50 & TRI50	ELE50 & WAS50	SET50 & SWP50	WAS & THOC2	SWP & RICE2
PAR25 & TRI75	ELE25 & WAS75	SET25 & SWP75	WAS & THOC3	SWP & RICE3
PAR0 & TRI100	ELE0 & WAS100	SET0 & SWP100	WAS & THOC4	SWP & RICE4

Note: 0, 25, 50, 75, and 100 are the levels of fresh green forage in the diet; 0, 1, 2, 3, and 4 are the levels of paddy rice (% BW). PAR - Para grass, TRI - Trichantera leaves, ELE - elephant grass, WAS - water spinach, SWP - sweet potato vines, SET - Setaria grass, and RICE - paddy rice.

Table 3. Variation in the nutrient contents of the experimental diets

Nutrients	n	Mean	SD	Cv%	Range
Energy, *Mcal ME kg^{-1} DM*	25	2.251 ± 0.276	0.269	11.73	1.905 ÷ 2.982
Protein, *% DM*	25	15.94 ± 3.98	4.81	29.42	10.39 ÷ 26.51
ADF, *% DM*	25	26.17 ± 4.58	4.69	18.57	13.16 ÷ 31.60

Data collection

All animals were individually weighed at the beginning of the experiment and thereafter once a week at 7:00 am before the morning feeding. The ADG was calculated by means of the slope of the linear regression of the weekly live weights on the experimental time.

During the experimental period, the offered feeds were weighed before each meal and the refusals were collected and weighed each morning before the first meal every day. Samples of the offered feeds and refusals were collected and dried at 105℃ until a constant weight to determine dry matter and then ground through a 1mm screening mesh (Cyclotec 1093 sample mill, Foss, Hillerød, Denmark). After a week, all daily feed and refusal samples were mixed (for each batch of feed offered and for refusals of each individual rabbit) for later analysis of chemical composition.

In the middle of the experiment, total feed intake and feces of each rabbit were collected over a period of 7 consecutive days. Representative fecal samples (10%) were taken daily from the total collected feces and stored at -25°C. On the last day of the collection period, the samples were bulked for each individual rabbit.

The samples of feeds and refusals, as well as fecal samples, were analyzed for dry matter, organic matter, ash, crude protein, and fat according to AOAC (1990). Neutral detergent

fiber (NDF) and ADF were determined following the procedures of Van Soest *et al.* (1991). ME was estimated based on chemical composition using the formulae of Lebas (2013).

Feed intake was calculated by the difference between the amount of feed offered and refusals on a dry matter (DM) basis. Digestibility (%) was calculated as (A-B/A)*100; where A and B are the total nutrient intake and total nutrient in the feces, respectively. The FCR was calculated as a ratio of DM intake per live weight gain.

Statistical analysis

In order to describe the nutrient-response curves, the generalized least squares model was applied to find the regression equations which best showed the responses of the rabbits (ADG and FCR) as dependent variables to the levels of diet nutrients (ME, CP, and ADF) as independent variables. After that, solver analysis was used to calculate the optimal levels of ME, CP, and ADF in the diet based on the following principles (Mercer, 1992; Vedenov & Pesti, 2008; Tedeschi *et al.*, 2010): (1) The optimal level of the nutrient input is the level that gives the maximal output level, which was applied for the ADG; and (2) The optimal level of the nutrient input is the lowest level calculated for each unit of output, which was applied for the FCR.

Results and Discussion

Table 3 and **Figures 1-3** show the parameters and patterns of regression of the ADG and FCR of the rabbits on the levels of ME, CP, and ADF in their diet. It can be seen that the level of ME in the diet had a significant influence on the ADG and FCR following the quadratic equations. The accuracy of the regression equation was *"high"* for the ADG and *"acceptable"* for the FCR. The ADG gradually increased with increasing levels of ME up to 2162 Kcal kg^{-1} DM, then decreased afterwards. In contrast, the FCR gradually decreased with increasing ME up to 2106 Kcal kg^{-1} DM, then increased. Combining the two curves, it can be seen that an energy level from 2106 to 2162 Kcal ME kg^{-1} DM was most suitable for the NZW growing rabbits.

The level of CP in the diet significantly affected the ADG and FCR following the quadratic functions with an *"average"* accuracy for both equations. The ADG tended to increase with increased CP up to 15%, and remained steady with 15-17% CP. With higher levels of CP, the ADG tended to decrease. Solver analysis found that the best CP level in the diet was 16.52% for the ADG. The FCR followed a reversed quadratic manner with the lowest value when the CP was 16.75%. So, with these two concerns, NZW growing rabbits best responded to diets with 16.50-16.75% CP on a DM basis.

Table 3. Regressions of the ADG and FCR of rabbits on the levels of ME, CP, and ADF in their diet

Regression equation	MPE	RPE	R^2	R^2adj
Level of ME, *Kcal kg^{-1} DM*				
(1) ADG = -73.00 + 0.08648ME - 0.000020ME²	1.44	7.10	77.10	76.50
(2) FCR = 33.24 - 0.02528 ME + 0.000006 ME²	0.60	11.01	52.30	51.06
Level of CP, *% DM*				
(3) ADG = 2.538 + 2.153CP - 0.06518CP²	2.80	13.82	69.90	69.20
(4) FCR = 11.78 - 0.7912CP + 0.02361CP²	0.67	12.38	50.80	49.90
Level of ADF, *% DM*				
(5) ADG = -23.99 + 4.12ADF - 0.0919ADF²	1.30	6.43	83.00	82.60
(6) FCR = 16.58 - 1.076ADF + 0.02461ADF²	0.58	10.74	51.90	50.90

Note: MPE: Mean prediction error, RPE: Relative prediction error, R^2: Coefficient of determination, R^2adj: Adjusted coefficient of determination.
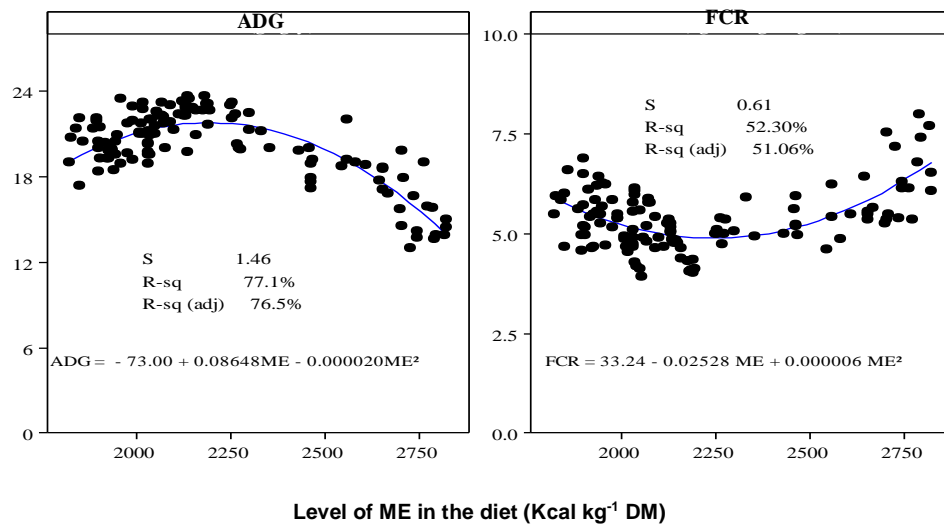
Figure 1. Response curves (ADG and FCR) of rabbits for the levels of ME in their diet
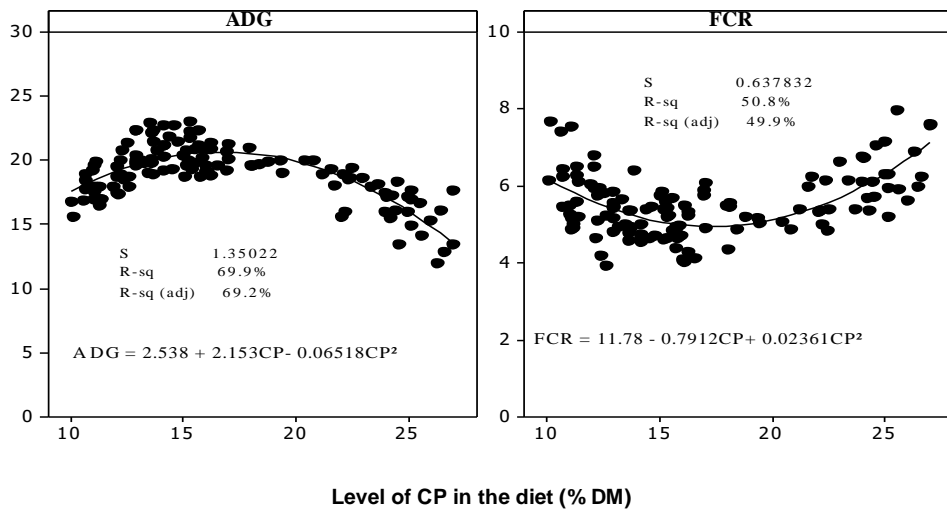

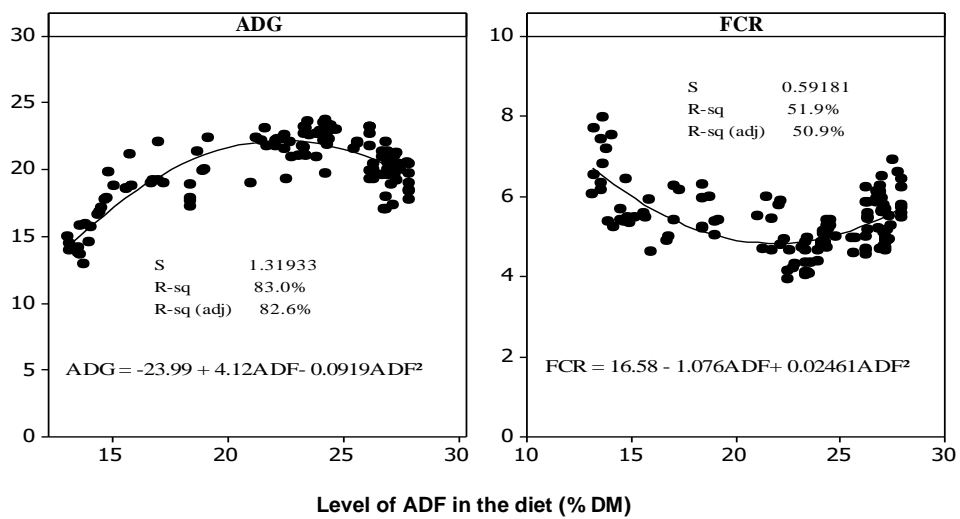**Figure 2.** Response curves (ADG and FCR) of rabbits for the levels of CP in their diet

Figure 3. Response curves (ADG and FCR) of rabbits for the levels of ADF in their diet

Both the ADG and FCR were also influenced by the level of ADF in the diet following quadratic manners with a "high" accuracy for the ADG and an "average" accuracy for the FCR. The ADG of the rabbit gradually increased and the FCR gradually decreased when the ADF was increased up to 22-23% DM, then a reversal trend happened. Solver analysis found that the ADG was highest when the diet contained 22.4% ADF and the FCR was lowest when the ADF was 21.86%.

The recommended level of energy for NZW growing rabbits is variable in the literature. Ali *et al.* (2011) showed that NZW male rabbits reached an ADG from 27.11 to 29.63 g day^{-1} when they were fed a diet with 2055 Kcal ME kg^{-1} DM. Obinne & Okorie (2008) stated that a diet with 9.7 Mj DE kg^{-1} DM (1901 Kcal ME kg^{-1} DM) could ensure the growth of rabbits in the tropics. However, according to Obinne & Mmereole (2010), the ADG of NZW rabbits was highest when the diet contained 10.8 Mj DE (2116 Kcal ME). On the other hand, Wang *et al.* (2012) saw that the suitable level of energy for NZW rabbits at 4-11 weeks of age was 11.7 Mj DE (2293 Kcal ME). NRC (1977) and Lebas & Gidenne (2000) recommended that the suitable energy level for rabbits was 2050 and 2187 Kcal ME kg^{-1} DM, respectively. According to the nutrient-response curves in our present study, the optimal level of energy (2106-2162 Kcal ME kg^{-1} DM) is in agreement with those recommended by NRC (1977), Lebas & Gidenne (2000), and Obinne & Mmereole (2010). This confirms the applicability of the method of mathematically modelling nutrient-response curves as a convenient way for the determination of nutrient requirements of rabbits.

The quadratic patterns of response of the ADG and FCR to CP may be due to the idea that at the beginning of the experiment the protein requirement was better met when CP was increased up to 16.75%, but not thereafter when the supply was more than needed by the animals. Dong *et al.* (2006) also found that the replacement of para grass, which has a low protein level (12.9%), in the basal diet with water spinach, which has a high protein level

(26.3% CP), clearly increased the ADG of crossbred rabbits. Luyen & Preston (2012) reported that sweet potato vines and paddy rice supported higher growth rates and better feed conversion than guinea grass (*Panicum maximum*) plus concentrates. This was probably due to the higher protein contents of the sweet potato vines and paddy rice feeding system (16.8-23.8% CP) compared with guinea grass plus concentrates system (11.5-14.3% CP). The authors also reported the best ADG (20.1 g d^{-1}) was obtained from a diet containing 60% sweet potato vines and 40% paddy rice (16.8% CP). Chat *et al.* (2005), who replaced guinea grass (low CP) with water spinach (high CP), found similar responses. However, having a too high a protein level was not necessary because the rabbits had to eliminate the excessive nitrogen from their bodies with some cost of energy when their protein requirement was already met. This may be the reason why in the present study the ADG tended to decrease when the level of CP in the diet was too high (>16.75%).

Ali *et al.* (2011), Obinne & Okorie (2008), and Obinne & Mmereole (2010) state that growth performance was at the highest rate when rabbits were fed diets with a CP content of 16%, 16%, and 16.2%, respectively. Similarly, Wang *et al.* (2012) concluded that the suitable content of CP for NZW rabbits at 4-11 weeks of age was 16%. The best CP level according to the present nutrient-response models (16.52-16.75% CP) seemed to be a little bit higher than the findings of the mentioned authors. This might be related to the lower quality of the tropical feeds used.

De Blas *et al.* (1999), Leng (2008), and De Blas & Wiseman (2010) state that fiber plays an important role in balancing the activities of microorganisms in the rabbit digestive system, maintaining intestine peristalsis, and thus helping to maintain a healthy digestive system. Moreover, fiber is a prominent resource of energy for microorganisms in the large intestine. If a diet is short in fiber, it would lead to a decrease in intestine peristalsis and it would take a longer time to circulate feed in the digestive system (Irlbeck, 2001). Nevertheless, having a too high level of fiber would lead to a

decrease in the digestion rate and activities of the microorganisms, which should cause a reduction in feed intake, ME, and other available nutrients in the diet. This would cause reduced productivity of the rabbit (Tao & Li, 2006; Osho *et al.*, 2013). Luyen & Preston (2012) found the best ADG when rabbits were fed a diet containing 60-70% sweet potato vines and 30-40% paddy rice, that contained 20.8-21.9% ADF. According to Pinheiro *et al.* (2009), the best level of ADF for rabbit growth was 23.3%. Osho *et al.* (2013) concluded that rabbits could reach a good growth rate when the diet contained 18.8-25.2% ADF. So, the best responses of rabbits to the level of ADF in the present models (21.86-22.42% ADF) are in accordance with their findings.

Based on the present nutrient-response models, the optimal levels of ME, CP, and ADF may be suggested for NZW growing rabbits fed on local forages to obtain the highest ADG and lowest FCR. However, the regression analyses were only made separately for each independent variable (nutrient) with an assumption that there was no interaction among them. Another important issue is that, so far, there have been few measurements of true ME in rabbits and most values are based on equations derived from other species. In the present study, the ME values of the diets were calculated based on such equations, so the results might be misleading, as they did not take into account the nature of the fiber component and specific digestive physiology of the rabbit (Leng, 2008). Thus, combinations of cereal-based concentrates and grasses may be less efficiently used than a diet based on highly digestible foliage such as sweet potato foliage or water spinach (Luyen & Preston, 2012). Furthermore, nutritional optima for the best animal performance are not necessarily economic optima as these levels will depend on the relative costs of the feed ingredients. To maximize profits, it is necessary to optimize, but not necessarily maximize, animal productivity.

Conclusions

The method of mathematically modelling nutrient-response curves can be conveniently applied to estimate the optimal levels of ME, CP, and ADF in the diets of NZW growing rabbits. It can, therefore, be preliminarily concluded from the present study that the best diet for NZW growing rabbits fed on available forages in the North of Vietnam contains 2106-2162 Kcal ME kg^{-1} DM, 16.5-16.8% CP, and 21.9-22.4% ADF. However, there is still a need for further research to confirm these findings, especially, when the interactions among the diet components are taken into account. In addition, the economic response should also be considered to ensure economic optima of practical diets.

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