PERFORMANCE AND BLOOD PARAMETERS OF RABBITS FED DIETS CONTAINING DECREASING LEVELS OF ALFALFA HAY

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ABSTRACT

Alfalfa hay is a fibrous food widely used in rabbit breeding, but its high cost and seasonal availability lead to the need for studies to evaluate its reduction in diet, without reducing nutrient availability and animal performance. This study aimed to evaluate the effect of decreasing alfalfa hay levels in rabbit diets on performance and blood parameters. For this purpose, an experiment was carried out using fifty New Zealand White rabbits of 31 days of age (25 males and 25 females) distributed, in a completely randomized design, in five treatments, with ten replicates per treatment (5 males and 5 females). Treatments were based on five decreasing levels of alfalfa hay in diet (26.72; 20.04; 13.36, 6.68 and 0.00%), with alfalfa hay reduction being offset by soybean meal and wheat bran. The first level of alfalfa inclusion (26.72%) was determined according to the amount generally used in commercial feeds. Animals were individually housed in metabolism cages with automatic drinking nipples. Throughout the experimental period (from 31 to 70 days of age), feed and water were provided *ad libitum*. The diets provided, the leftovers and the animals were weighed at the beginning of the experiment (31 days), at 50 days, and at the end of the experiment (70 days) to determine the performance variables. At the end of the experimental period, blood biochemical parameters were determined (glucose, total proteins, urea, triglycerides, total cholesterol, HDL and LDL). From 31 to 50 days of age, rabbits fed diets containing decreasing levels of alfalfa hay presented a linear reduction in final weight ($P<0.001$). For the phase of 31 to 70 days of age, there was a quadratic effect ($P=0.013$) of alfalfa hay level on final weight, with the highest value being reached at 25.37%. There was also a quadratic effect ($P=0.048$) of alfalfa hay level on weight gain, with the best response estimated at 25.64%. There was no variation ($P<0.05$) in blood biochemical parameters of rabbits fed diets containing decreasing alfalfa hay levels. While decreasing alfalfa hay inclusion in the diet from 26.72 to 0.00% had no effect on blood biochemical parameters of growing rabbits, the highest weight gain from 31 to 70 days was obtained with 25.64% of alfalfa hay.

Key words: Fibre source, Legume forage, Rabbit feeding

INTRODUCTION

The feeding of rabbits produced in commercial systems usually employs alfalfa hay due to its high content of acid detergent fibre (34%) and crude protein (18%) (Pompeu *et al.*, 2003). However, because it has a high price and variable market availability, it can represent up to 40% of the diets cost (Scapinello *et al.*, 2003), compromising the manufacture of feed when alfalfa cultivation is harmed by climate variations. In this sense, studies aiming to replace alfalfa hay become important (Retore *et al.*, 2010), but caution is needed not to compromise the availability of nutrients or productive performance of animals. In Brazil, as in many countries, the most commonly used protein ingredient is soybean meal, due to its high biological protein value and regularity on the market, which makes it the main protein concentrate in monogastric diets (Dávila *et al.*, 2007). Another food with huge potential for the gradual replacement of alfalfa hay, associated with soybean meal, is the wheat bran, as it has an appreciable crude protein content (14 to 16%) and also contains more acid detergent fibre than soybean meal (13 to 15%) (Ferreira *et al.*, 2015). The present work aimed to evaluate the performance and blood biochemical parameters of rabbits fed diets containing decreasing alfalfa hay levels, replaced by soybean meal and wheat bran.

MATERIALS AND METHODS

Animals, diets and experimental design

The experiment was carried out in the Rabbit Breeding Sector of State University of Maringá, located in Paraná State, Brazil (23°21'S, 52°04'W and altitude of 564 m). The entire experimental procedure was previously approved by the Committee of Ethical Conduct for Experimental Animal Use. Fifty 31-day old New Zealand White rabbits (25 males and 25 females) were distributed, following a completely randomized design, in five treatments, with ten replicates per treatment (5 males and 5 females). Treatments were based on five decreasing levels of alfalfa hay in diet (26.72; 20.04; 13.36, 6.68 and 0.00%), with alfalfa hay reduction being offset by soybean meal and wheat bran. The first level of alfalfa inclusion (26.72%) was determined according to the amount generally used in commercial feeds. Animals were individually housed in metabolism cages with automatic drinking nipples. The experimental diets were formulated based on corn, soybean meal, alfalfa hay, wheat bran, amino acids, minerals and vitamins to fulfil the requirements for growing rabbits (De Blas and Mateos, 2010), as expressed in Table 1. After mixing the ingredients, the feed was pelletized using a Commercial Industry Pelletizer (Chavantes™, model 40 HP) with capacity from 800 to 1,700 kg/h, with a 4.5 mm matrix and without addition of steam, at an average temperature of 70°C (60 to 80°C) for about 50 seconds.

Table 1: Experimental diets for growing rabbits with decreasing levels of alfalfa hay (natural matter)

Ingredients	Levels of alfalfa hay (%)				
	26.72	**20.04**	**13.36**	**6.68**	**0.00**
Corn (maize)	30.00	28.87	27.82	26.77	25.73
Soybean meal, 46% CP	6.22	6.38	6.63	6.88	7.14
Wheat bran	34.56	42.16	49.51	56.83	64.16
Alfalfa hay	26.72	20.04	13.36	6.68	0.00
Limestone	0.888	1.275	1.466	1.642	1.818
Dicalcium phosphate	0.348	0.024	0.000	0.000	0.000
DL-Methionine (98.5%)	0.108	0.091	0.075	0.058	0.042
L-Lysine HCl (78.5%)	0.204	0.193	0.182	0.170	0.158
Mineral and vitamin premix[1]	0.500	0.500	0.500	0.500	0.500
Salt	0.400	0.400	0.400	0.400	0.400
Coccidiostatic[2]	0.060	0.060	0.060	0.060	0.060
Chemical composition calculated					
Dry matter (%)	88.63	88.59	88.56	88.53	88.50
Crude protein (%)	15.40	15.40	15.40	15.40	15.40
Digestible energy (Mcal/kg)	2.40	2.40	2.40	2.40	2.40
Acid detergent fibre (%)	16.00	15.84	14.64	13.45	12.25
Calcium (%)	0.800	0.800	0.800	0.800	0.800
Total phosphorus (%)	0.570	0.570	0.624	0.682	0.739
Methionine + Cysteine (%)	0.590	0.590	0.590	0.590	0.590
Lysine (%)	0.780	0.780	0.780	0.780	0.780

[1]Premix provided per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 1,000 IU; vitamin E acetate, 50 mg; vitamin K_3, 2 mg; biotin, 0.1 mg; Fe, 100 mg; Cu, 20 mg; Mn, 50 mg; Co, 2 mg; I, 1 mg; Zn, 100 mg; Se, 0.1 mg; BHT, 100 mg; [2] Robenidine, 66 mg.

Performance

Throughout the experimental period (from 31 to 70 days of age), feed and water were provided *ad libitum*. Feed provided, leftovers and animals were weighed at the beginning of the experiment (31 days), at 50 days, and at the end of the experiment (70 days) in order to calculate feed intake, weight gain and feed conversion. To calculate the production cost of the treatments (in American dollars, U$), the average prices of the inputs from Maringá-Brazil were used. The production cost of diets per kilogram of live weight gain (Yi) was determined, according to Bellaver *et al.* (1985), as follows:
Yi = (Qi x Pi) / Gi , where:

Yi = feed cost per kilogram of live weight gain for the i-th treatment;
Pi= price per kilogram of feed used for the i-th treatment;
Qi= amount of feed intake for the i-th treatment;
Gi= weight gain for the i-th treatment;

Blood parameters

At 70 days of age, the animals fasted for 4 hours prior to blood collection, which was done through the jugular vein. Blood was transferred to tubes containing heparin or fluoride, and then centrifuged at 3,000 rpm during 15 minutes for plasma separation. After that, 3 mL of plasma was transferred to properly identified Eppendorf tubes and stored in a freezer (-18 °C) for further analysis of glucose, total protein, urea, triglycerides, total cholesterol, HDL and LDL, performed by colorimetric method using commercial kits.

Statistical analysis

The UNIVARIATE procedure was applied to evaluate the presence of outliers. The normality of experimental errors and the homogeneity of variances between treatments for the various variables were previously evaluated using the Shapiro-Wilk and Levene tests (SAS, 2010), respectively. Analysis of variance (ANOVA) was performed using the procedure General Linear Models of the statistical software SAS (SAS Inst. Inc., Cary, NC, USA). For performance variables, the initial weight was used as a covariate. The degrees of freedom regarding the alfalfa hay reduction levels in the diets were split into orthogonal polynomials to obtain the regression equations, according to the best fit. For all analyses, a significance level (P) of 0.05 was adopted.

RESULTS AND DISCUSSION

Rabbits fed diets containing decreasing levels of alfalfa hay from 31 to 50 days of age presented a linear reduction (P<0.001) in final weight (Table 2a). For the phase of 31 to 70 days of age (Table 2b), there was a quadratic effect (P=0.013) of alfalfa hay level on final weight, with the highest value being reached at 25.37%. There was also a quadratic effect (P=0.048) of alfalfa hay level on weight gain, with the best response estimated at 25.64%. There was no variation (P<0.05) in blood biochemical parameters of rabbits fed diets containing decreasing alfalfa hay levels (Table 3), which indicates that isoproteic and isoenergetic diets with higher levels of soybean meal and wheat bran in substitution for alfalfa hay do not alter the availability of circulating nutrients. Although the experimental diets were isoproteic and isoenergetic, there was variation in the level of acid detergent fibre, which decreased from 16.00 to 12.25% among diets, and in the level of total phosphorus, which increased from 0.570 to 0.739%. Results obtained for the growth performance may be due to these nutritional variations in the diets. Even so, plasma levels of total protein and urea did not differ significantly among diets (P>0.05), indicating thus that there were no differences in circulating amino acid levels or protein catabolism, respectively. It means that all diets satisfactorily met rabbit amino acid requirements (Ferreira *et al.*, 2015). Rabbits perform caecal fermentation, which allows them to modulate the synthesis of nutrients such as amino acids, vitamins and short-chain fatty acids. By means of caecotrophy, rabbits can ingest some of the pre-faecal material, as caecotrophs directly derive from caecal fermentation. This mechanism results in more efficient utilization of relatively nutrient- or fibre-poor diets (Arruda *et al.*, 2003). In the present study, all these factors may have resulted in nutrient compensation, so that even the lowest dietary level of acid detergent fibre did not result in a decrease of the blood biochemical parameters evaluated.

Table 2a: Performance of growing rabbits fed diets containing decreasing levels of alfalfa hay from 31 to 50 days of age

Variables	Levels of alfalfa hay (%)					SEM[1]	P-value
	26.72 (n=10)	20.04 (n=10)	13.36 (n=10)	6.60 (n=10)	0.00 (n=10)		
Initial weight (g)	631	675	581	610	544	-	-
Final weight (g) [2]	1367	1467	1215	1293	1201	17.49	<0.001
Weight gain (g/d)	36.82	39.62	31.71	34.16	32.87	0.87	0.187
Feed intake (g/d)	82.19	87.43	75.72	82.68	70.43	1.94	0.171
Feed conversion	2.24	2.23	2.50	2.84	2.14	0.16	0.708
Production cost (U$/kg gained)	0.72	0.67	0.71	0.76	0.66	0.05	0.688

Table 2b: Performance of growing rabbits fed diets containing decreasing levels of alfalfa hay from 31 to 70 days of age

Variables	Levels of alfalfa hay (%)					SEM[1]	P-value
	26.72 (n=10)	**20.04 (n=10)**	**13.36 (n=10)**	**6.60 (n=10)**	**0.00 (n=10)**		
Initial weight (g)	631	675	581	610	544		
Final weight (g) [3]	2168	2391	2038	2070	1954	22.61	<0.001
Weight gain (g/d) [4]	38.44	42.91	36.43	36.50	35.26	0.56	0.016
Feed intake (g/d)	81.55	89.45	74.96	81.88	77.26	1.41	0.156
Feed conversion	2.12	2.10	2.07	2.31	2.19	0.05	0.774
Production cost (U$/kg gained)	0.68	0.64	0.59	0.62	0.58	0.04	0.178

1- Standard error of mean.
2- Linear effect (P-value = 0.012): $Y= 7.5679x + 1207.70$ (r^2=0.72).
3- Quadratic effect (P-value = 0.013): $Y= 0.4675x^2 + 23.722x + 1932.50$ (r^2=0.75).
4- Quadratic effect (P-value = 0.048): $Y= 0.0078x^2 + 0.40x + 34.65$ (r^2= 0.70).

Table 3: Blood biochemical parameters of growing rabbits fed diets containing decreasing levels of alfalfa hay

Variables	Levels of alfalfa hay (%)					SEM[1]	P-value
	26.72 (n=10)	20.04 (n=10)	13.36 (n=10)	6.60 (n=10)	0.00 (n=10)		
Glucose (mg/dL)	83.60	92.90	96.40	102.40	82.50	4.80	0.651
Total protein (g/dL)	6.31	6.38	6.94	5.93	6.17	0.17	0.532
Urea (mg/dL)	39.50	33.00	34.63	32.30	36.00	0.77	0.061
Triglycerides (mg/dL)	54.40	92.83	83.56	73.15	79.84	5.01	0.208
Total cholesterol (mg/dL)	79.13	82.81	67.20	65.96	63.48	2.70	0.132
HDL (mg/dL)	26.00	24.20	23.00	24.60	24.30	0.65	0.744
LDL (mg/dL)	68.90	65.50	57.75	78.30	71.20	2.93	0.345

1- Standard error of mean.

CONCLUSIONS

Even though the decrease of alfalfa hay level in diet from 26.72 to 0.00% did not affect the blood biochemical parameters of growing rabbits, the highest weight gain from 31 to 70 days was obtained with 25,64% of alfalfa hay.

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