

EFFECT OF COWPEA (*VIGNA UNGUICULATA* (L.) WALP.) STOVER DIETARY INCLUSION LEVEL ON TOTAL TRACT APPARENT DIGESTIBILITY OF NUTRIENTS IN GROWING RABBITS

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Abstract: Although agro-industrial co-products have low economic value as foods for human consumption, they may have potential value as animal feedstuffs. This experiment was carried out to evaluate the effect of cowpea stover inclusion in rabbits' diet on growth performance and nutrient digestibility. A total of 180 animals were randomly assigned to 3 treatments (CS0, CS2 or CS4, with no inclusion, 20 or 40 g/kg of cowpea stover, respectively). Animal performance was evaluated between the 53rd and 67th d of age in 48 animals per treatment. The coefficients of total tract apparent digestibility (CTTAD) of organic matter, crude protein, neutral detergent fibre and gross energy were measured between 63 to 67 d of age in 12 animals per treatment. Results showed that, in general, CTTAD values were not affected by the inclusion of cowpea stover. Nevertheless, a trend towards a decrease in crude protein digestibility ($P=0.0848$) was observed when including cowpea stover. This had a negative influence on digestible protein ($P=0.0240$) and on the ratio between digestible protein and digestible energy ($P=0.0231$) for diet CS4. Rabbits showed normal figures for growth rate (on av. 46.8 g/d), feed intake (on av. 168.3 g/d) and feed conversion ratio (on av. 3.61). Future studies should assess the possibility of incorporating higher levels of cowpea stover while analysing the economic impact of this inclusion.

Key Words: digestibility, legume co-products, nutrition, performance, rabbit.

INTRODUCTION

Different high fibre content feed ingredients are frequently used in rabbit feeds, e.g. alfalfa hay, grape seed, olive leave, paprika meal, soybean hull, sunflower hull and wheat straw (García *et al.*, 2000; Nicodemus *et al.*, 2007; Ribeiro *et al.*, 2012). Alfalfa (*Medicago sativa*) hay is the preferred fibre source and is usually included up to 20-40% in feeds. However, the rising prices of ingredients frequently used in animal feeding has led to the evaluation of alternative and less costly ingredients, as feed can account for up to 70% of the total costs of rabbit production (Gidenne *et al.*, 2017). Although it could be argued that feed costs could be minimised by using cheaper, non-traditional feed ingredients, the inclusion of these feed resources is limited, as most of the times data on their nutritive value are scarce and highly variable (Lima *et al.*, 2017; de Blas *et al.*, 2018; Uhlířová *et al.*, 2018). Furthermore, possible effects on animal growth performance must also be evaluated.

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<https://doi.org/10.4995/wrs.2019.10450>

Cowpea (*Vigna unguiculata*) is grown primarily for human nutrition and feeding. Its production results in large amounts of mature plants, with high fibre content, which are generally discarded as organic waste or can be partially used in animal feeding (Gonçalves *et al.*, 2016). A source of dietary fibre is essential to prevent digestive disorders in growing rabbits and several classes of fibre, including low-digested and digestible fibre, have been recommended for use in rabbit feeds (Gidenne, 2003).

Although it is accepted that cowpea stover may have potential for use as animal feed (Anele *et al.*, 2011, 2012; Samireddypalle *et al.*, 2017), to the authors' knowledge no studies have been conducted using it as a feed ingredient in rabbit diets.

This study aimed to assess the effect of the inclusion of different levels of cowpea stover on the performance and nutrient digestibility of growing rabbits.

MATERIALS AND METHODS

Animals and diets

The trial was carried out in the animal facility of the ESTIRPE - Estirpe D'Honra, Unipessoal Lda at Bragança, Portugal, between November and December of 2014. New Zealand×Californian rabbits were kept in a closed air-conditioned building maintained between 18 and 23°C and received 12 h of light daily (07:00 to 19:00 h). Rabbits were handled according to the Portuguese legislation (Ports No. 1005/92, 214/08, and 635/09) on animal welfare. Rabbits were weaned at 35 d of age and between this date and the beginning of the trial (53 d of age) were fed a commercial diet.

Cowpea stover (comprising the aerial part after grain harvest) was collected after harvesting of cowpea grains in Famalicão, Portugal. The cowpea stover was dried and chopped into lengths of 1-2 cm and stored in a cool environment until analysis, then used as raw material to manufacture the experimental diets (Table 1). Three dietary treatments (isoenergetic and isonitrogenous) were formulated according to De Blas and Mateos (2010) to meet the requirements of rabbits at fattening stage. The 3 diets were prepared by including 20 or 40 g/kg (CS2 and CS4, respectively) of cowpea stover into a basal diet (CS0), as presented in Table 1. In CS2 and CS4 diets, the alfalfa hay, beet pulp and wheat straw were partially replaced by cowpea stover and wheat bran. During the experiment, the rabbits had access to feed and water *ad libitum* and neither the diets nor the water contained any drug supplementation.

Experimental design, growth performance and in vivo digestibility

A total of 180 rabbits of both sexes at 53 d of age and similar live weight (on av. 1816 g,) were reared until slaughter (67 d of age) and the growth performance and nutrient digestibility were measured. One of the reasons why the experimental period started when animals reached 53 d of age is related to the higher efficiency in fibre digestibility in older animals (Ribeiro *et al.*, 2012). The second reason is related to the risk of mortality caused by mucoid enteropathy, which is generally high up to 50 d of age (Bennegadi *et al.*, 2003; Martínez-Paredes *et al.*, 2009).

For each experimental diet, growth performance was measured in 48 rabbits distributed into 16 collective cages (3 rabbits per cage). During the experimental period, individual live weight and feed intake per cage were recorded and the weight gain, daily feed intake and feed conversion ratio were calculated. It should be noted that the mortality rate was not calculated, as only 4 animals died during the trial.

After the first 10 d of the experiment, samples of faeces and experimental diets were collected during 4 d (63 to 67 d of age) from another 36 rabbits (12 per treatment), housed in individual cages from the onset of the experiment (53 d of age), to determine *in vivo* digestibility according to the European reference method (Perez *et al.*, 1995).

Chemical analysis

Samples of the experimental diets and faeces were dried in an air-forced oven at 50°C and ground to 1 mm sieve prior to chemical analysis. AOAC (1990) procedures were used to determine dry matter, (DM; 934.01), organic matter (OM; 942.05), crude protein (CP; 954.01), crude fibre (962.09) and ether extract (920.39). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin fractions were obtained by the detergent methodologies without the use of sodium sulphite and expressed exclusive of ash (Robertson and Van Soest, 1981; Van Soest *et al.*, 1991). The acid

Table 1: Ingredients and chemical composition of cowpea (*Vigna unguiculata*) stover (CS) and experimental diets containing 0 (CS0), 20 (CS2) or 40 g/kg (CS4) of cowpea stover.

Ingredients (g/kg, as fed)	Experimental diets			
	CS	CS0	CS2	CS4
Cowpea stover		0	20	40
Alfalfa hay		223	218	213
Sunflower meal		200	200	200
Wheat		150	150	150
Beet pulp		103	93	83
Wheat bran		85	90	95
Wheat straw		66	56	46
Corn gluten feed		35	35	35
Palm kernel		35	35	35
Defatted grape seed		30	30	30
Soybean oil		18	18	18
Colza meal		10	10	10
Sugarcane molasses		10	10	10
Minerals, vitamins and additives ¹		35	35	35
Chemical composition (g/kg, as fed)				
Dry matter	917	923	916	921
Organic matter	836	821	817	828
Crude protein	127	153	151	150
Crude fibre	303	213	213	216
Starch	11	83	74	73
Neutral detergent fibre	541	383	387	393
Acid detergent fibre	432	226	244	246
Lignin	83	62	70	71
Acid detergent-insoluble nitrogen	2.5	2.4	2.1	2.3
Ether extract	-	29	27	28
Gross energy (MJ/kg DM)	17.5	18.1	18.3	18.2

¹Calcium carbonate, Luctarom 1408-Z, Sepiolite, NL-510-R, salt, Biolys 70, Sodium bicarbonate and Bio-Mos.

detergent-insoluble nitrogen (ADIN) was determined according to Goering and Van Soest (1990). Total starch was quantified using an enzymatic assay procedure (K-TSTA-100A, Megazyme, Ireland). Gross energy was determined in an adiabatic oxygen bomb calorimeter (Parr 6300, Moline, IL, USA).

Statistical analysis

Data analysis was performed using the SAS 9.2 program (SAS, 2009). The effect of the inclusion of different levels of cowpea stover on growth performance and *in vivo* digestibility was analysed by one-way ANOVA. For multiple mean comparisons, the Tukey test was used. Significant differences were set up at $P < 0.05$ and trends were considered when 10% ($P < 0.10$).

RESULTS AND DISCUSSION

Cowpea stover showed high fibre (541 g/kg; NDF) and a reasonable concentration of protein (127 g/kg; CP). Results on chemical composition of legume straws are rather scarce when compared to cereal straws. Nevertheless, data presented by Bruno-Soares *et al.* (2000) evaluating the chemical composition of seven different legume straws reported NDF values varying from 580 to 765 g/kg DM. Additionally, López *et al.* (2005) showed that for 11 legume straws NDF fraction varied from 454 to 669 g/kg DM. Although NDF values of cowpea straw are within the reported

variation, the data on CP concentration (127 g/kg) are higher than the values reported by those authors, ranging from 43 to 114 g/kg DM. Foster *et al.* (2009), evaluating cowpea hay, also reported a relative lower value of CP, averaging 117 g/kg DM. Nevertheless, Anele *et al.* (2011, 2012) reported CP values around 190 g/kg DM. It is well known that the maturity stage of plants will influence the nutritive value of forages and fibrous feedstuffs. However, in the case of cereal straws, typically harvested when the grain evenly dries to the desired moisture concentration, NDF and CP contents are not that variable. For legume stovers, as pulses are collected when 80-90% of the pods reach the appropriate maturity stage and the quantity of immature pods and grains that remain in the residue might be quite variable, variations in NDF and CP contents could be significant. Furthermore, as flowering time (Catt and Paull, 2017) and number of flowers (Gray and Brady, 2016) are influenced by genotype and environmental conditions, thus conditioning the number of pods and their maturation, higher variation in the chemical composition of legume stovers is expected. This variation has been reported by Anele *et al.* (2011, 2012), who verified that cowpea haulms obtained from 6 varieties in the rainy season presented lower CP values ($P < 0.001$) than the haulms of the same varieties cultivated in the dry season (164 and 215 g/kg DM, respectively).

Altering the proportion of these ingredients in the diet to obtain 20 (CS2) and 40 g/kg (CS4) of cowpea stover inclusion did not change the coefficients of total tract apparent digestibility (CTTAD) of OM, CP, NDF and gross energy (Table 2). However, a trend ($P = 0.0848$) towards lower CP digestibility with the increase of cowpea stover inclusion was detected, with a decrease from 68.9 to 64.4% as the level of cowpea inclusion increased from 0 to 40 g/kg (CS4). This variation could be the result of the change in the dietary protein quality linked to the variation in the ingredients (Villamide *et al.*, 2010). Furthermore, the indigestible protein concentrations could also have influenced CP digestibility. Although several authors have reported negative correlations between ADIN and CP digestibility (Martínez and Fernández, 1980; Villamide and Fraga, 1998) our data show no variation within the ADIN concentrations of the diets, thus excluding this hypothesis. On the other hand, changes in the fibre constituents (compared to CS0, ADF and lignin increased in CS2 and CS4) could also have contributed to reducing CP digestibility by affecting the passage rate through the gastrointestinal tract (Gidenne, 2003). This negative influence was clear for the data on digestible protein (DP) and for the data on the ratio between DP and digestible energy (DE), so that higher inclusion levels of cowpea stover had a negative impact on DP ($P = 0.0240$) and on DP/DE ($P = 0.0231$) values.

The DP contents were below the recommended values for growth (101 vs. 105-110 g/kg) and the digestible energy (DE) contents were above the recommended values (10.9 vs. 10.0-10.5 MJ/kg) proposed by Xiccato and Trocino (2010). Consequently, the DP to DE ratio of the experimental diets was lower than the recommended values (9.3 vs. 10.5-11.0 g/MJ) proposed by the same authors. Nevertheless, cowpea stover inclusion in the diets did not affect the growth performance (live weight at the 67 d, daily weight gain, daily feed intake and feed conversion rate), which remained within normal values (Table 3).

Table 2: Daily feed intake, coefficients of total tract apparent digestibility (CTTAD) and dietary nutritive value digestibility of the experimental diets with different levels of cowpea (*Vigna unguiculata*) stover (CS) in growing rabbits between 63 to 67 d of age. n = 12 rabbits (individual cage) per experimental diet.

	Experimental diets ¹			SEM	P-value
	CS0	CS2	CS4		
Average daily feed intake (g/d)	173.5	180.6	187.3	6.36	0.2417
CTTAD (%)					
Organic matter	56.3	56.8	55.3	1.98	0.8557
Crude protein	68.9	67.7	64.4	1.68	0.0848
NDF	30.8	29.1	27.3	2.58	0.5750
Gross energy	56.3	55.2	54.5	2.14	0.7623
Dietary nutritive value					
Digestible protein (DP) (g/kg)	105.4 ^b	101.3 ^{ab}	96.6 ^a	2.73	0.0240
Digestible energy (DE) (MJ/kg)	11.0	11.0	10.8	0.42	0.8343
Ratio DP/DE (g/MJ)	9.6 ^b	9.2 ^{ab}	9.0 ^a	0.20	0.0231

¹CS0, CS2 and CS4 diets containing 0, 20 or 40 g/kg (as fed) of cowpea stover.

SEM: standard error of the mean. Values with different letters within a line differ significantly by the Tukey test ($P < 0.05$).

Table 3: Growth performance of rabbits fed on the experimental diets with different levels of cowpea (*Vigna unguiculata*) stover (CS), n = 48 rabbits (16 cage) per experimental diet.

	Experimental diets ¹			SEM	P-value
	CS0	CS2	CS4		
Live weight (g)					
53 d	1820	1828	1802	35.9	0.8593
60 d	2172	2158	2155	36.6	0.9318
67 d	2495	2447	2474	36.6	0.5926
Daily weight gain (g/d)					
53-60 d	50.2	47.1	50.4	2.23	0.4873
60-67 d	46.2	41.3	45.6	2.36	0.2728
53-67 d	48.2	44.2	48.0	1.78	0.1964
Daily feed intake (g/d)					
53-60 d	152.1	149.6	152.3	7.42	0.3696
60-67 d	182.0	184.6	189.3	6.61	0.3819
53-67 d	167.0	167.1	170.8	5.59	0.3698
Feed conversion rate					
53-60 d	3.03	3.18	3.02	0.203	0.9802
60-67 d	3.94	4.46	4.15	0.253	0.2851
53-67 d	3.47	3.78	3.57	0.140	0.3268

¹CS0, CS2 and CS4, diets containing 0, 20, or 40 g/kg (as fed) of cowpea stover, respectively; SEM, standard error of mean.

CONCLUSION

Cowpea stover may present good potential for use in rabbit feeding. However, protein digestibility values indicate that its inclusion should be further evaluated so that possible detrimental effects of the fibre fraction can be overcome. In this way, chemical or biological pre-treatments of cowpea stover should also be considered, as they might improve the digestibility of this feedstuff.

Acknowledgements: This work received support by the European Project EUROLEGUME (Seventh Research Framework Programme of the European Union – FP7 research project no. 613781) and by European Investment Funds by FEDER/COMPETE/POCI–Operational Competitiveness and Internationalisation Programme, under Project POCI-01-0145-FEDER-006958 and National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019. Ederson Andrade is recipient of a predoctoral fellowship from the CAPES Foundation (grant number BEX-13521/13-6).

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