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EFFECT OF FEED RESTRICTION AND DIFFERENT CRUDE PROTEIN SOURCES ON THE PERFORMANCE, HEALTH STATUS AND CARCASS TRAITS OF GROWING RABBITS

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Abstract: We evaluate the effect of the crude protein source (CP) in combination with 2 feeding strategies (AL: *ad libitum* or R: restricted) on the performance, health status and carcass traits of growing-fattening rabbits (between 33 and 75 d of age). Two experimental diets were formulated; the first containing soybean meal (SBM: 70 g/kg) as the main CP source and the second containing white lupin seeds (WLS: 105 g/kg) as the main CP source. A total of 160 weaned Hyplus rabbits (774±10 g live weight) were randomly allocated to the experimental groups, combination of feeding strategy and diet (AL SBM, R SBM, AL WLS and R WLS; 40 rabbits per group with 4 rabbits per cage), for 42 d. The feeding programme was applied as follows: both the AL SBM and AL WLS rabbits were fed *ad libitum* for the entire fattening period, whereas the R SBM and R WLS rabbits were subjected to feed restriction between 33 and 47 d of age (75% of the *ad libitum* intake). Afterwards, all restricted rabbits were fed *ad libitum* until 75 d of age. Regardless of the CP source, the feed restriction reduced the final live weight by 83 g, chilled carcass weight by 65 g, and dressing out percentage by 0.9 percentage points (P<0.05). There was a higher chilled carcass weight (+114 g; P=0.001) and reference carcass weight (+91 g; P=0.001) in rabbits fed with WLS diet than in rabbits fed with SBM diet. No dead or morbid rabbits were observed in restricted rabbits during the restriction period. There was no effect of the diet on the health of rabbits. However when the rabbits of AL SBM and AL WLS group were compared did we observe a lower number of rabbits at sanitary risk in AL WLS group (2 *vs.* 12 rabbits, P=0.006). In conclusion, the growth performance was not affected by the crude protein source, and no interaction between dietary CP source and feeding regime was observed. Feed restriction regime did not improve sanitary risk index throughout the entire period.

Key Words: carcass quality, digestive health, feeding regime, growth, protein source, rabbit.

INTRODUCTION

Digestive disorders are the main pathological events affecting weaned or fattening rabbits (Rosell, 2003; Rosell *et al.*, 2009) and despite great breakthroughs in diagnostic techniques and the adoption of better farm practices (i.e. sanitation, hygiene control, feed restriction), the use of antibiotics as a prevention practice is common (Agnoletti, 2012). As the European Union banned the use of antibiotics in feeds as growth promoters (EC Council, 2003), there is increasing interest in finding alternative strategies to reduce or even avoid the use of antibiotics to control the incidence of enteropathies (Le Bouquin *et al.*, 2013).

Maertens (2007) reviewed some possibilities to reduce antibiotic use in rabbit meat production, including suitable management techniques. Among them, “all-in all-out” production systems, together with proper disinfection and cleaning, as well as using minimal disease reproduction stock, are promising tools allowing a reduction in use of antimicrobials through better farm hygiene standards. In addition to management approaches, the search for new feeding strategies to increase rabbit resistance to digestive pathologies is becoming a primary focus of research (De

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Blas *et al.*, 2012). In this regard, a suitable dietary crude protein source (CP) for intensively reared broiler rabbits appears to be an important factor. Traditionally used soybean meal (SBM) has a favourable effect on the growth rate and feed efficiency of rabbits, but may increase the risk of digestive disorders in weaned rabbits (Carabaño *et al.*, 2009).

Recently, Volek *et al.* (2014) reported that one suitable dietary CP source for growing-fattening rabbits, as a replacement of SBM, is white lupin seeds (WLS; *Lupinus albus* cv. Amiga). In addition to CP, lupins have a unique carbohydrate composition characterised by negligible levels of starch, high levels of soluble and insoluble non-starch polysaccharides and high levels of raffinose oligosaccharides (Van Barneveld, 1999; Volek and Marounek, 2009; Volek *et al.*, 2013), all of which can affect the rabbit's digestive health in a beneficial way (Gidenne *et al.*, 2010). Furthermore, the lipid fraction of WLS is rich in essential fatty acids, linoleic and alpha-linolenic, respectively, which makes WLS very interesting from a nutritional point of view (Volek and Marounek, 2011; Chiofalo *et al.*, 2012; Volek *et al.*, 2014). Previous authors have suggested a possible use for lupin seed as a nutraceutical feed to improve the nutritional quality of animal products (Volek and Marounek, 2011; Chiofalo *et al.*, 2012).

Another way to limit digestive disorders in weaned rabbits may be the application of a post-weaning feed intake limitation strategy (Gidenne *et al.*, 2012; Knudsen *et al.*, 2014). Knudsen *et al.* (2015) showed that post-weaning quantitative feed restriction modulated gut immunity in growing rabbits. However, there are some contradictory results in the literature regarding the favourable effects of feed restriction in the digestive health of young rabbits in terms of the entire fattening period (Birolo *et al.*, 2013). Furthermore, the favourable effects of lower intake on health did not persist when rabbits were fed *ad libitum* (Gidenne *et al.*, 2003, 2009a,b; Romero *et al.*, 2010), and no significant effect of a limited feed intake on the digestive health of growing-fattening rabbits was observed (Martignon *et al.*, 2009). Apparently, different management strategies used in terms of the duration and type of feed restriction may explain contradictory results cited (Maertens, 1992). In addition, several disadvantages of feed restriction, such as lower final live weight and dressing-out percentage, have been observed in restricted rabbits (Maertens, 1992; Gidenne *et al.*, 2009a,c; Knudsen *et al.*, 2013, 2014). Thus, the post-weaning feed intake limitation strategy should be further studied in relation to the growth performance and carcass traits, as well as the digestive health of rabbits, in terms of the entire fattening period. In addition, information regarding the interaction of dietary CP source and feed restriction on the growth and digestive health of growing rabbits is scarce. Therefore, our aim was to evaluate the effect of the CP source (SBM or WLS) in combination with the feeding regime (*ad libitum* or restricted) on the performance and carcass traits of fattening rabbits. Sanitary status is also reported.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of the Institute of Animal Science and the Central Commission for Animal Welfare at the Ministry of Agriculture of the Czech Republic (Prague, Czech Republic) and was conducted according to the guidelines for applied nutrition experiments in rabbits (Fernández-Carmona *et al.*, 2005).

Experimental diets

Ingredients and chemical composition of experimental diets, formulated following the recommendations of De Blas and Mateos (2010), are shown in Table 1. The SBM diet contained 70 g/kg of soybean meal as the main CP source, whereas the WLS diet contained 105 g/kg of white lupin seeds as the main CP source. The experimental diets included no dietary fat or synthetic amino acids and had similar CP, digestible protein (DP), starch and digestible energy (DE) contents. The experimental diets also had a standard fibre concentration to avoid nutritionally induced enteropathy in the growing rabbit (Bennegadi-Laurent *et al.*, 2013) the type and proportions of fibre fractions being similar between diets. Regardless of the higher ether extract of WLS diet, due to a relatively high ether extract content of WLS, the DE content of SBM and WLS diets was similar. The diets were offered as 3-mm pellets that were 5 to 10 mm long. No antibiotics were included in the feed or in the drinking water. The only dietary inclusion was a coccidiostat (66 mg of robenidine hydrochloride/kg of feed; Alpharma, Belgium).

Table 1: Ingredients and chemical composition of the experimental diets containing soybean meal (SBM) or white lupin seeds (WLS) as the main crude protein sources.

	White lupin seeds	SBM diet	WLS diet
Ingredient (g/kg)			
Alfalfa meal		300	300
Soybean meal (48% CP)		70	0
White lupin seeds		0	105
Wheat bran		330	330
Sugar beet pulp		70	50
Oats		150	125
Barley		50	60
Vitamin supplement[1]		9.93	9.93
Dicalcium phosphate		5	5
Limestone		10	10
Salt		5	5
Robenidine hydrochloride		0.07	0.07
Analysed composition (g per kg as-fed basis)			
Dry matter	884	889	881
Crude protein	348	153	148
Neutral detergent fibre	303	375	366
Acid detergent fibre	211	183	187
Lignin	89	39	47
Ether extract	92	37	43
Starch		162	157
Calculated values[2]			
Digestible protein (DP, g)		114	110
Digestible energy (DE, MJ)		10.2	9.8
DP/DE (g/MJ)		11.2	11.2

[1]Provides the following quantities per kg of complete diet: vitamin A (retinol), 12000 IU; vitamin D3 (cholecalciferol), 2000 IU; vitamin E (α-tocopherol), 50 mg; vitamin K3 (bisulphate menadione complex), 2 mg; vitamin B1 (thiamine mononitrate), 3 mg; vitamin B2 (riboflavin), 7 mg; vitamin B6 (pyridoxine), 4 mg; niacinamide, 50 mg; Ca-pantothenate, 20 mg; folic acid, 1.7 mg; biotin, 0.2 mg; vitamin B12 (cyanocobalamin), 0.02 mg; choline chloride, 600 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 20 mg; Fe (as $FeSO_4 \cdot 7H_2O$), 50 mg; I (as KI), 1.2 mg; Mn (as MnO), 47 mg; Zn (as ZnO), 50 mg; Se (as Na_2SeO_3), 0.15 mg.
[2]Calculated from the values obtained in the digestibility trial.

Animals and experimental design

The study was conducted at an experimental rabbit farm at the Institute of Animal Science (Prague, Czech Republic), which is accredited in accordance with European Union standards. The building was equipped with a forced ventilation and cooling system. Animals were maintained under room temperature varying between 18°C and 20°C, with relative humidity of 65% and a photoperiod of 12 h of light and 12 h of dark.

A total of 160 Hyplus rabbits (774±10 g live weight), 33 d old at the beginning of the trial, were randomly allocated to one of 4 experimental groups (AL SBM, R SBM, AL WLS and R WLS; 40 rabbits per group) and fed one of the 2 experimental diets (SBM diet or WLS diet) for 42 d, at which time the rabbits were 75 d old. The rabbits were housed in wire net cages (80×60×45 cm), 4 per cage. The feeding programme was applied as follows: both the AL SBM rabbits and AL WLS rabbits were fed *ad libitum* for the entire fattening period, whereas the R SBM and R WLS rabbits were restrictively fed from 33 to 47 d of age (during the first 14 d after weaning). Afterwards, all restricted rabbits were fed *ad libitum* until the end of the fattening period (from 47 to 75 d old).

During the feed restriction period, restricted rabbits were fed at a level of 75% of the *ad libitum* groups. Briefly, feed intake was recorded daily in the morning (between 7:00 and 8:00 h) during the entire fattening period; thus, the restrictive feed ration was calculated every day for the restriction period, based on the previous average daily feed intake of the rabbits fed *ad libitum*. Rabbit live weight (weekly) and feed intake (daily) were recorded per cage, and average daily weight gain, average feed intake and feed conversion ratio were calculated afterwards.

The sanitary risk index of rabbits was calculated according to the definitions of the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005), as the sum of morbid and dead rabbits, given that each animal was considered only once (i.e., classified as either dead or morbid). Mortality was checked daily, and morbidity was assessed weekly through an individual control of all clinical signs of digestive troubles. Morbidity corresponds to sick rabbits (remaining alive within the experimental period) exhibiting digestive troubles (diarrhoea, mucus in faeces or abnormal caecotrophy, i.e., exhibiting a high amount of uneaten soft faeces for more than 3 d) or severe weight loss for 1 wk (animals not included in the previous categories but exhibiting an abnormally low growth rate). An animal was considered morbid only once, even if diarrhoea lasted several days. Cages with a mortality rate higher than 50% were not included in the statistical analysis of growth performance (Gidenne *et al.*, 2009a). In fact, 1 cage (3 rabbits died, 1 rabbit remaining alive) for the AL SBM and R SBM experimental group, and 2 cages (3 rabbits died per cage, 1 rabbit remaining alive in each cage) for the R WLS were excluded from the performance evaluation.

At the end of the experiment, 15 rabbits/group were randomly selected, weighed and slaughtered without previous fasting in an authorised abattoir next to the Institute of Animal Science; these rabbits were used to assess the carcass traits according to the harmonised criteria and terminology for rabbit meat research (Blasco and Ouhayoun, 1996). Briefly, the slaughtered rabbits were stunned and bled, and then the skin, genitals, urinary bladder, gastrointestinal tract and distal part of the legs were removed. Carcasses were weighed (hot carcass weight) and then chilled at +4°C for 24 h in a ventilated room. After chilling, the chilled carcasses were weighed (chilled carcass weight). The head, thoracic cage organs, liver and kidneys were removed from each carcass to obtain the reference carcass weight, which includes the meat, bones and fat depots.

Digestibility trial

Twenty weaned Hyplus rabbits (33 d of age, 954±76 g live weight) were used in a digestibility trial to determine the coefficients of total tract apparent digestibility (CTTAD) for dry matter, CP, gross energy, starch and neutral detergent fibre of the experimental diets, following the European Group on Rabbit Nutrition guidelines (Perez *et al.*, 1995). The rabbits were individually housed in digestibility cages (50×40×42.5 cm) and randomly assigned to one of the 2 experimental diets (10 rabbits/diet). Diets were offered *ad libitum* and after an adaptation period of 27 d, the daily feed intake and total faeces collection were recorded (from 60 to 64 d of age).

Chemical analytical methods

Chemical analysis of diets, white lupin seeds and dried faeces for ether extract, starch, dry matter, CP and acid detergent fibre (ADF) were determined according to the official methods of analysis 920.39, 920.40, 934.01, 954.01 and 973.18 of AOAC (AOAC, 2005). Neutral detergent fibre (NDF), exclusive of residual ash, was assayed with heat-stable amylase (Mertens, 2002), lignin levels were determined by solubilisation of cellulose with sulphuric acid (Robertson and Van Soest, 1981) and gross energy was determined in adiabatic calorimeter (C5000 control, IKA-Werke, Staufen, Germany).

Statistical analysis

Data regarding the growth performance were evaluated using a mixed linear model considering repeated measurements. Parameters were estimated by the REML method using a MIXED procedure from the Statistical Analysis System (SAS, 2003), taking into consideration as main effects the diet (SBM or WLS), the feeding regime (*ad libitum* or restricted) and their interaction. In the model, random (co)variances between days of age intervals were summarised by residual R matrix, which was assumed to be a block diagonal with identical submatrices, each corresponding to an individual cage. Carcass traits were analysed in a two-way ANOVA using the GLM procedure of SAS. The main factors were the diet (SBM or WLS) and feeding regime (*ad libitum* or restricted) and their interaction. For growth performance traits, cage was the experimental unit and for carcass traits the individual rabbit represented the experimental unit. Additionally, analysis of growth performance traits considered data from morbid animals as recommended by Gidenne *et al.* (2009a). Data regarding the coefficients of total tract apparent digestibility of dry matter, CP, gross energy, NDF and starch were examined by one-way ANOVA considering the diet (SBM or WLS) as fixed effect. The individual rabbit was used as the experimental unit. The results are presented as means followed by the standard error mean. Means were compared by the Scheffe test. Health status was assessed using Fisher's Exact Test. The individual rabbit was used as the experimental unit. All differences were considered to be significant at $P<0.05$.

RESULTS

The CP source did not affect the live weight, feed intake, average daily gain or feed conversion ratio (Table 2). During the feed restriction period (from 33 to 47 d of age), the limited feed intake reduced the weight gain by 11.0 g/day (P=0.001) and consequently, at 47 d of age, restricted rabbits weighted 161.0 g less than rabbits fed *ad libitum* for the entire fattening period (P=0.001). When returning to *ad libitum* intake (in terms of the whole period between 48 and 75 d of age), no significant differences were observed in the average daily gain (P=0.504), and we did not observe a better feed conversion ratio in restricted rabbits (P=0.128). Over the entire fattening period, the feed restriction reduced the feed consumption (by 6.4 g/d. P=0.003) and final live weight of rabbits at 75 d of age (by 83 g. P=0.028) but did not affect the feed conversion ratio (AL: 3.05 *vs.* R: 3.11 g/kg of feed). No significant interaction between the dietary CP source and feeding regime was observed in terms of rabbit growth performance.

The carcass characteristics were greatly affected by the feeding regime (Table 3). The feed restriction reduced the chilled carcass weight by 65 g, reference carcass weight by 63 g and dressing out percentage by 0.9 percentage points ($P\leq0.041$). Restricted rabbits also showed a greater full digestive tract weight (+10 g) and higher drip loss percentage (0.54 percentage points) than rabbits fed *ad libitum* ($P\leq0.006$). The CP sources also affected the carcass characteristics. There was a higher full digestive tract weight (+8 g), chilled carcass weight (+114 g) and reference

Table 2: Live weight, daily feed intake, average daily gain and feed conversion ratio of fattening rabbits[1] fed according to the diet (SBM: soybean meal diet or WLS: white lupin seed diet) and the feeding regime (AL: *ad libitum* or R: restricted).

Diet	SBM		WLS			*P*-value		
Feeding Regime	AL	R	AL	R	SEM	CP source	Feeding Regime	CP source × Feeding Regime
Live weight (g)								
At 33 d[2]	769	775	788	764	20	0.802	0.602	0.405
At 47 d	1461	1327	1527	1339	30	0.157	0.001	0.328
At 75 d	2903	2827	2952	2863	40	0.240	0.028	0.857
Daily feed intake (g)								
33 to 47 d	103.0	77.7	108.1	79.6	2.7	0.224	0.001	0.382
48 to 75 d	178.3	182.8	180.6	185.5	3.1	0.362	0.094	0.934
33 to 75 d	152.6	146.9	155.9	149.0	2.2	0.170	0.003	0.758
Average daily gain (g/d)								
33 to 47 d	49.4	39.4	52.8	41.1	1.8	0.126	0.001	0.599
48 to 75 d	39.0	36.9	39.3	39.3	1.7	0.379	0.504	0.501
33 to 75 d	50.8	47.3	51.4	51.2	1.7	0.141	0.227	0.288
Feed conversion (g feed/g of live weight)								
33 to 47 d	2.18	2.12	2.13	2.01	0.11	0.413	0.338	0.751
48 to 75 d	4.65	5.02	4.64	5.03	0.27	0.990	0.128	0.954
33 to 75 d	3.04	3.15	3.06	3.07	0.14	0.810	0.624	0.681

[1]36, 36, 39 and 32 animals for the AL SBM experimental group (rabbits fed a diet based on SBM *ad libitum* for the entire fattening period), R SBM experimental group (rabbits fed a diet based on SBM with feed restriction from 33 to 47 d of age), AL WLS experimental group (rabbits fed a diet based on WLS *ad libitum* for the entire fattening period) and R WLS experimental group (rabbits fed a diet based on WLS with feed restriction from 33 to 47 d of age), respectively.
[2]At weaning.
SEM: Standard error of means.

Table 3: Carcass characteristics of fattening rabbits fed according to the diet (SBM: soybean meal diet or WLS: white lupin seed diet) and the feeding regime (AL: *ad libitum* or R: restricted).

Diet	SBM		WLS			*P*-value		
Feeding Regime	AL	R	AL	R	SEM[3]	CP source	Feeding Regime	CP source × Feeding Regime
Number of animals	15	15	15	15				
Slaughter weight (g)	2955	2967	3135	3054	21	0.001	0.362	0.221
Skin weight (g)	155	151	150	148	1	0.019	0.078	0.699
Full digestive tract weight (g)	163	172	171	181	2	0.015	0.006	0.916
Chilled carcass weight (g)	1719	1711	1889	1768	18	0.001	0.041	0.070
Reference carcass weight (g)	1386^{b}	1379^{b}	1532^{a}	1414^{b}	15	0.001	0.020	0.041
Drip loss percentage[1]	2.46	2.92	2.28	2.89	0.07	0.437	0.001	0.563
Perirenal fat (g)	17	18	19	21	1	0.101	0.403	0.634
Dressing out percentage[2]	58.1	57.5	58.9	57.8	0.2	0.128	0.026	0.448

[1]Drip loss percentage=[(hot carcass weight–chilled carcass weight)/hot carcass weight]×100.

[2]Dressing out percentage=(chilled carcass weight/slaughter weight)×100.

SEM: Standard error of means.

[a,b]Means in the same row with different superscripts differ significantly at $P<0.05$.

carcass weight (+91 g) with a lower skin weight (–4 g) in rabbits fed with WLS diet than rabbits fed with SBM diet ($P\leq0.019$). There was an interaction between the CP source and feeding regime for the reference carcass weight ($P=0.041$). Thus, the highest reference carcass weight was observed in the rabbits of AL WLS group compared to the other groups.

No dead or morbid rabbits were observed in restricted rabbits during the restriction period. After the restriction period when rabbits were fed *ad libitum*, the sanitary risk index was impaired. When an analysis of the restricted feeding regime between weeks 1 and 2 against weeks 3 to 6 was performed, the Fisher's Exact Test for this comparison resulted in a $P<0.05$ for the sanitary health index. Thus, feed restriction regime did not improve sanitary risk index throughout the entire period (Table 4). Similarly, we did not observe any effect of the diet on rabbit mortality, morbidity or the sanitary risk index. Only when the rabbits from the AL SBM and AL WLS groups were compared did we observe both a lower morbidity (1 *vs.* 9 rabbits; $P=0.014$) and sanitary risk index (2 *vs.* 12 rabbits; $P=0.006$) in the AL WLS rabbits compared with the AL SBM rabbits. Mortality and morbidity were associated with diarrhoea.

The CTTAD of the dry matter, CP, gross energy, or NDF of rabbits fed the different experimental diets did not differ (Table 5). However, the CTTAD of starch was higher in rabbits fed the diet based on SBM than in rabbits fed the diet based on WLS by 0.011 ($P=0.005$).

Table 4: Number of rabbits housed, dead (mortality), morbidity and at sanitary risk (sanitary risk index) according to the diet (SBM: soybean meal diet or WLS: white lupin seed diet) or the feeding regime (AL: *ad libitum* or R: restricted).

	Diet			Feeding Regime		
	SBM	WLS	*P*-value	AL	R	*P*-value
No. of rabbits						
Housed	80	80		80	80	
Dead	7	7	1.219	4	10	0.160
Morbid	13	6	0.141	10	9	1.000
Sanitary risk index[1]	20	13	0.241	14	19	0.435

[1]Sanitary risk index=number of dead+number of morbid; (morbid animals that died was count as dead).

Table 5: Coefficients of total tract apparent digestibility for the diets containing soybean meal (SBM diet) or white lupin seeds (WLS diet) as the main crude protein sources.

	Diet			
	SBM	WLS	SEM	*P*-value
Apparent digestible coefficient				
Dry matter	0.621	0.628	0.006	0.608
Crude protein	0.747	0.746	0.007	0.924
Gross energy	0.635	0.633	0.005	0.811
Starch	0.974	0.963	0.002	0.005
Neutral detergent fibre	0.340	0.350	0.012	0.691
Daily feed intake (g)	217.8	208.8	4.6	0.339

SEM: Standard error of means (n=10 rabbits/diet).

DISCUSSION

Health status of rabbits

Mortality, morbidity and sanitary risk index were assessed as complementary information, as the sample size limits the extrapolation of our results. Nevertheless, we observed a positive impact of feed restriction on the sanitary risk index during the restriction period. However, during the re-alimentation period our results suggest an increase of the number of rabbits at sanitary risk. Gidenne *et al.* (2003; 2009a,b) did not find a greater sanitary risk index for restricted rabbits when the restriction period ended. The main difference between our study and those carried out by these authors was in the way rabbits were re-fed. While we fed animals *ad libitum* after the restricted period, Gidenne *et al.* (2003; 2009a) performed an adaptation of animals from restricted to *ad libitum* intake, and Gidenne *et al.* (2009b) applied a longer restriction period, allowing animals on the restricted groups to feed *ad libitum* at older ages. These methodological differences should be taken into account when adopting different feeding regimes. Reduced morbidity and sanitary risk index were observed in the rabbits in the AL WLS group for the entire fattening period than in rabbits in the AL SBM group, which is consistent with Volek *et al.* (2014). However, the favourable effect of the R WLS treatment on the digestive health of rabbits, after returning to an *ad libitum* intake, was not observed. Further studies should be conducted to elucidate this observation.

Growth performance

In the present study, the protein source did not affect the weight gain, feed intake or feed conversion ratio, which confirms our previous reports regarding white lupin seeds as a suitable dietary CP source replacement of soybean meal in rabbit diets (Volek and Marounek, 2009; Volek and Marounek, 2011; Volek *et al.*, 2014).

Regardless of the CP sources, feed restriction had negative effects on both the weight gain and final live weight that are consistent with the reports of Xiccato (1999), Gidenne *et al.* (2009a; 2009c) and Knudsen *et al.* (2013; 2014). Contrary to the results of Gidenne *et al.* (2009a) and Knudsen *et al.* (2014), we did not observe a favourable effect of the post-weaning limited intake strategy on the feed conversion ratio; this was most likely associated with the different finishing weight of rabbits or the duration and type of feed restriction (Maertens, 1992). In fact, other authors (Perrier, 1998; Romero *et al.*, 2010), who used the feeding regime similar to that of the present study (2 or 3-wk restriction of 20 or 30% in rabbits weaned at 35 d of age), also found that feed conversion ratio of restricted rabbits was similar to that of rabbits fed continuously *ad libitum* in terms of the entire fattening period.

Carcass characteristics

In the present study, feed restriction had a negative effect on the chilled carcass weight, reference carcass weight, drip loss percentage and dressing-out percentage, even if the slaughter weights of rabbits used for the carcass traits were not different among groups. This is consistent with the findings of Gondret *et al.* (2000) and generally concurs with the reviews describing a relationship between feed restriction and carcass quality (Xiccato, 1999; Gidenne *et al.*, 2012). In our study, the lower dressing-out percentage of restricted rabbits was most likely associated with the lower carcass weight in restricted rabbits, the higher full digestive tract weight and the reduced growth rate, which enhances the relative growth of early-maturing tissues, in particular those of the digestive tract (Lebas and Laplace, 1982; Ouhayoun, 1998; Chodová and Tůmová, 2013). In our study, no effect of the feed restriction was observed on the proportion of perirenal fat, which agrees with the findings of Gidenne *et al.* (2009a).

In the present study, there was a higher chilled and reference carcass weight in rabbits fed with WLS diet than in rabbits fed with SBM diet. In other species, for example lambs, Ponnampalam *et al.* (2002), who compared at the same isonitrogenous level 2 protein sources (lupin and fish meal) differing in rumen degradation rate and in lipid composition, and observed that lupin diet resulted in heavier carcases than the fish meal or barley/fish meal diets. The previous observation was then confirmed by Ponnampalam *et al.* (2003). The authors concluded that these findings suggest that rapidly degradable protein and energy from lupin was more efficiently utilised for the carcass gain than that obtained from fish meal. Although there are no available data on the comparison of ileal apparent digestibility of soybean meal and lupin protein in terms of anti-nutritional factors such as trypsin inhibitory activity, the findings of Gutiérrez *et al.* (2003) suggest that the different protein sources (soybean meal, sunflower meal, soybean and potato protein concentrates) in isonitrogenous diets of young rabbits may affect ileal apparent digestibility of protein, even if apparent faecal digestibility of crude protein was not affected by the protein source. In this context, further studies should be conducted to elucidate whether there is a difference between ileal apparent digestibility of soybean meal and lupin seeds in growing-fattening rabbits, as well as whether this aspect is able to affect carcass gain. In the present study, in spite of the higher full digestive tract weight in rabbits fed the WLS diet than that of rabbits fed with SBM diet, we did not observe a decrease of dressing out percentage. These results may be partially explained by the lower skin weight recorded in rabbits fed the lupin diet. As dressing out percentage was not affected by dietary treatments, we did not observe any difference in adipose tissue (Ouhayoun *et al.*, 1986). Volek and Marounek (2009) reported a higher dressing out percentage in rabbits fed the diet based on WLS than in rabbits fed with SBM diet. These contradictory results may be related to a lower full digestive tract weight of rabbits fed the WLS diet than that of rabbits fed the SBM in the study of Volek and Marounek (2009), unlike the rabbits fed the WLS diet in the present study. Furthermore, there was also a lower slaughter weight of rabbits used for carcass characteristics determination (on av. 2684 g) in the work of Volek and Marounek (2009) than in the present study (on av. 3028 g).

Total tract apparent digestibility of experimental diets

There was a lower CTTAD of starch in rabbits fed the WLS diet, which was most likely due to the slightly higher ADF/starch ratio of this diet (Gidenne *et al.*, 2000).

CONCLUSION

The growth performance was not affected by the crude protein source, and no interaction between dietary CP source and feeding regime was observed. Feed restriction had a negative effect on the finial live weight and carcass traits, regardless of the CP source. Feed restriction regime did not improve sanitary risk index throughout the entire period.

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REFERENCES

Agnoletti F. 2012. Update on rabbit enteric diseases: Despite improved diagnostic capacity, where does disease control and prevention stand? *In Proc.: 10th World Rabbit Congress, September 3-6, 2012. Sharm-El-Sheikh, Egypt, 1113-1127.*

AOAC International. 2005. Official methods of analysis of the Association of Official Analytical Chemists, 18th ed. *AOAC, Gaithersburg, MD, USA.*

Bennegadi-Laurent N., Licois D., Gidenne T. 2013. Nutritionally induced enteropathy in the growing rabbit: impact on caecal microbial activity and blood metabolic profile. *Rev. Med. Vet., 164: 495-502.*

Birolo M., Tazzoli M., Majolini D., Trocino A., Xiccato G. 2013. Effect of feed restriction level and period on performance and health of growing rabbits. *In Proc.: Giornate di Coniglicoltura ASIC 2013, April 10-11, 2013. Fiera di Forlì, Italy, 57-59.*

Blasco A., Ouhayoun J. 1996. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Sci., 4: 93-99. doi:10.4995/wrs.1996.278*

Carabaño R., Villamide M.J., García J., Nicodemus N., Llorente A., Chamorro S., Menoyo D., García-Rebollar P., García-Ruiz A.I., De Blas J.C. 2009. New concepts and objectives for protein-amino acid nutrition in rabbits: a review. *World Rabbit Sci., 17: 1-14. doi:10.4995/wrs.2009.664*

Chodová D., Tůmová E. 2013. The effect of feed restriction on meat quality of broiler rabbits: a review. *Sci. Agric. Bohem., 44: 55-62. doi:10.7160/sab.2013.440109*

Chiofalo B., Presti V.L., Chiofalo V., Gresta F. 2012. The productive traits, fatty acid profile and nutritional indices of three lupin (*Lupinus* spp.) species cultivated in a Mediterranean environment for the livestock. *Anim. Feed Sci. Tech., 171: 230-239. doi:10.1016/j.anifeedsci.2011.11.005*

De Blas C., Mateos G.G. 2010. Feed formulation. *In: The Nutrition of the Rabbit. 2nd ed., De Blas C., Wiseman J. (ed.). CABI International, Wallingford, UK, 222-232. doi:10.1079/9781845936693.0222*

De Blas J.C., Chamorro S., García-Alonso J., García-Rebollar P., García-Ruiz A.I., Gómez-Conde M.S., Menoyo D., Nicodemus N., Romero C., Carabaño R. 2012. Nutritional digestive disturbances in weaner rabbits. *Anim. Feed Sci. Tech., 173: 102-110. doi:10.1016/j.anifeedsci.2011.12.016*

EC Council. 2003. Regulation (EC) of the European Parliament and of the Council on additives for use in animal nutrition. *No. 1831/2003/EC, 22 September 2003. Off. J. Eur. Comm., 18 October 2003, L 268, 29-43.*

Fernández-Carmona J., Blas E., Pascual J.J., Maertens L., Gidenne T., Xiccato G., García J. 2005. Recommendations and guidelines for applied nutrition experiments in rabbits. *World Rabbit Sci., 13: 209-228. doi:10.4995/wrs.2005.516*

Gidenne T., Pinheiro V., Falcão e Cunha L. 2000. A comprehensive approach of the rabbit digestion: consequences of a reduction in dietary fibre supply. *Livest. Prod. Sci., 64: 225-237. doi:10.1016/S0301-6226(99)00141-4*

Gidenne T., Feugier A., Jehl N., Arveux P., Boisot P., Briens C., Corrent E., Fortune H., Montessuy S., Verdelhan S. 2003. Un rationnement alimentaire quantitatif post-sevrage permet de réduire la fréquence des diarrhées, sans dégradation importante des performances de croissance: résultats d'une étude multi-site. *In Proc.: 10émes Journées de la Recherche Cunicole, 19-20 November, 2003. Paris, France, 29-32.*

Gidenne T., Combes S., Feugier A., Jehl N., Arveux P., Boisot P., Briens C., Corrent E., Fortune H., Montessuy S., Verdelhan S. 2009a. Feed restriction strategy in the growing rabbit. 2. Impact on digestive health, growth and carcass characteristics. *Animal, 3: 509-515. doi:10.1017/S1751731108003790*

Gidenne T., Aymard P., Bannelier C., Combes S., Lamothe L. 2009b. Interaction entre la stratégie de restriction et la concentration énergétique de l'aliment: impact sur la croissance et la santé du lapin. Premiers résultats. *In Proc.: 13émes Journées de la Recherche Cunicole, 17-18 Novembre, 2009. Le Mans, France.*

Gidenne T., Travel A., Murr S., Oliveira H., Corrent E., Foubert C., Bebin K., Mevel L., Rebours G., Renouf B., Gigaud V. 2009c. Ingestion restreinte et mode de distribution de la ration. Conséquences sur le comportement alimentaire, la digestion et la qualité de la carcasse. *In Proc.: 13émes Journées de la Recherche Cunicole, 17-18 Novembre, 2009. Le Mans, France, 43-46.*

Gidenne T., García J., Lebas F., Licois D. 2010. Nutrition and feeding strategy: interactions with pathology. *In: The Nutrition of the Rabbit. 2nd ed., De Blas C., Wiseman J. (ed.). CABI International, Wallingford, UK, 179-199. doi:10.1079/9781845936693.0179*

Gidenne T., Combes S., Fortun-Lamothe L. 2012. Feed intake limitation strategies for the growing rabbit: effect on feeding behaviour, welfare, performance, digestive physiology and health: a review. *Animal, 6: 1407-1419. doi:10.1017/S1751731112000389*

Gondret F., Lebas F., Bonneau M. 2000. Restricted feed intake during fattening reduces intramuscular lipid deposition without modifying muscle fiber characteristics in rabbits. *J. Nutr., 130: 228-233.*

Gutiérrez I., Espinosa A., García J., Carabaño R., De Blas C. 2003. Effect of protein source on digestion and growth performance of early-weaned rabbits. *Anim. Res., 52: 461-471. doi:10.1051/animres:2003032*

Knudsen C., Combes S., Briens C., Duperray J., Rebours G., Salaun J-M., Travel A., Weissman D., Gidenne T. 2013. Ingestion restreinte et concentration énergétique de l'aliment: impact sur la santé, les performances et le rendement à l'abattage du lapin. *In Proc. : 15èmes Journées de la Recherche Cunicole, 19-20 Novembre, 2013. Le Mans, France, 47-50.*

Knudsen C., Combes S., Briens C., Coutelet G., Duperray J., Rebours G., Salaun J-M., Travel A., Weissman D., Gidenne T. 2014. Increasing the digestible energy intake under a restriction strategy improves the feed conversion ratio of the growing rabbit without negatively impacting the health status. *Livest. Sci., 169: 96-105. doi:10.1016/j.livsci.2014.08.015*

Knudsen C., Combes S., Briens C., Duperray J., Rebours G., Salaun J-M., Travel A., Weissman D., Gidenne T. 2015. Quantitative feed restriction rather than caloric restriction modulates the immune response of growing rabbits. *J. Nutr., 145: 483-489. doi:10.3945/jn.114.197871*

Lebas F., Laplace J.P. 1982. Mensurations viscérales chez le lapin. 4. Effets de divers modes de restriction alimentaire sur la croissance corporelle et viscérale. *Ann. Zootech., 31: 391-430. doi:10.1051/animres:19820403*

Le Bouquin S., Rouxel G., Mihoc E., Chauveau V., Terrade F., Chauvin C. 2013. Facteurs humains et usages des antibiotiques en filière cunicole: etude de quelques déterminants psychologiques. *In Proc.: 15émes Journées de la Recherche Cunicole, 19-20 Novembre, 2013. Le Mans, France, 115-119.*

Maertens L. 1992. Rabbit nutrition and feeding: a review of some recent developments. *J. Appl. Rabbit Res., 15: 889-913.*

Maertens L. 2007. Strategies for the reduction of antibiotic utilization during rearing. *In Proc.: Giornate di Coniglicoltura ASIC 2007, 26-27 September, 2007. Fiera di Forlì, Italy, 1-11.*

Martignon M.H., Combes S., Gidenne T. 2009. Rôle du mode de distribution de l'aliment dans une stratégie de rationnement: conséquences sur le profil d'ingestion, la croissance et la santé digestive du lapin. *In Proc.: 13émes Journées de la Recherche Cunicole, 17-18 Novembre, 2009. Le Mans, France, 39-42.*

Mertens D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J. AOAC. Int., 85: 1217-1240.*

Ouhayoun J., Lebas F., Delmas D. 1986. The effects of feeding regimes on growth and carcass quality in rabbit. *Cuni-Sciences, 3: 7-21.*

Ouhayoun J. 1998. Influence of the diet on rabbit meat quality. *In: The Nutrition of the Rabbit. De Blas C., Wiseman J. (ed.). CABI International, Wallingford, UK, 177-195.*

Perez J.M., Lebas F., Gidenne T., Maertens L., Xiccato G., Parigi-Bini R., Dalle Zotte A., Cossu M.E., Carazzolo A., Villamide M.J., Carabaño R., Fraga M.J., Ramos M.A., Cervera C., Blas E., Fernández J., Falcão e Cunha L., Bengala Freire J. 1995. European reference method for *in vivo* determination of diet digestibility in rabbits. *World Rabbit Sci., 3: 41-43. doi:10.4995/wrs.1995.239*

Perrier G. 1998. Influence de deux niveaux et deux durées de restriction alimentaire sur l'efficacité productive du lapin et les caractéristiques bouchéres de la carcasse. *In Proc.: 7émes Journées de la Recherche Cunicole, 17-18 May, 1998. Lyon, France, 179-182.*

Ponnampalam E.N., Sinclair A.J., Hosking B.J., Egan A.R. 2002. Effects of dietary lipid type on muscle fatty acid composition, carcass leanness, and meat toughness in lambs. *J. Anim. Sci., 80: 628-636.*

Ponnampalam E.N., Hosking B.J., Egan A.R. 2003. Rate of carcass components gain, carcass characteristics, and muscle longissimus tenderness in lambs fed dietary protein sources with a low quality roughage diet. *Meat Sci., 63: 143-149. doi:10.1016/S0309-1740(01)00202-9*

Robertson J.B., Van Soest P.J. 1981. The detergent system of analysis. *In: James W.P.T., Theander O. (ed.). The analysis of dietary fibre in food. Marcel Dekker, NY, 123-158.*

Romero C., Cuesta S., Astillero J.R., Nicodemus N., De Blas C. 2010. Effect of early feed restriction on performance and health status in growing rabbits slaughtered at 2 kg live-weight. *World Rabbit Sci., 18: 211-218. doi:10.4995/wrs.2010.778*

Rosell J.M. 2003. Health status of commercial rabbitries in the Iberian peninsula. A practitioner's study. *World Rabbit Sci., 11: 157-169. doi:10.4995/wrs.2003.505*

Rosell J.M., de la Fuente L.F., Badiola J.I., Fernández de Luco D., Casal J., Saco M. 2009. Study of urgent visits to commercial rabbit farms in Spain and Portugal during 1997-2007. *World Rabbit Sci., 17: 127-136. doi:10.4995/wrs.2009.652*

Statistical Analysis System. 2003. SAS/STAT User's Guide (Release 9.2). *SAS Inst. Inc. Cary NC, USA.*

Van Barneveld R.J. 1999. Understanding the nutritional chemistry of lupin (*Lupinus* spp.) seed to improve livestock production efficiency. *Nutr. Res. Rev., 12: 203-230. doi:10.1079/095442299108728938*

Volek Z., Marounek M. 2009. Whole white lupin (*Lupinus albus* cv. Amiga) seeds as a source of protein for growing-fattening rabbits. *Anim. Feed Sci. Tech., 152: 322-329. doi:10.1016/j.anifeedsci.2009.05.003*

Volek Z., Marounek M. 2011. Effect of feeding growing-fattening rabbits a diet supplemented with whole white lupin (*Lupinus albus* cv. Amiga) seeds on fatty acid composition and indexes related to human health in hind leg meat and perirenal fat. *Meat Sci., 87: 40-45. doi:10.1016/j.meatsci.2010.08.015*

Volek Z., Volková L., Marounek M. 2013. Effect of a diet containing white lupin hulls (*Lupinus albus* cv. Amiga) on total tract apparent digestibility of nutrients and growth performance of rabbits. *World Rabbit Sci., 21: 17-21. doi:10.4995/wrs.2013.1191*

Volek Z., Marounek M., Volková L., Kudrnová E. 2014. Effect of diets containing whole white lupin seeds on rabbit doe milk yield and milk fatty acid composition as well as the growth and health of their litters. *J. Anim. Sci., 92: 2041-2049. doi:10.2527/jas.2013-7120*

Xiccato G. 1999. Feeding and meat quality in rabbits: a review. *World Rabbit Sci., 7: 75-86. doi:10.4995/wrs.1999.383*