

## MEAT QUALITY OF RABBITS REARED WITH TWO DIFFERENT FEEDING STRATEGIES: WITH OR WITHOUT FRESH ALFALFA *AD LIBITUM*

CAPRA G.\*, MARTÍNEZ R.†, FRADILETTI F.\*, COZZANO S.†, REPISO L.‡, MÁRQUEZ R.‡, IBÁÑEZ F.\*

\*Instituto Nacional de Investigación Agropecuaria (INIA). Estación Experimental INIA Las Brujas. Ruta 48, Km.10, Rincón del Colorado, CANELONES CP 90200, Uruguay.

†Universidad Católica del Uruguay. Avda. 8 de Octubre 2738. CP 11600 MONTEVIDEO, Uruguay.

‡Laboratorio Tecnológico del Uruguay (LATU). Avda Italia 6201. CP 11400. MONTEVIDEO, Uruguay.

**Abstract:** The aim of this study was to evaluate production performance, carcass characteristics and nutritive value of meat of rabbits reared under the 2 prevailing feeding strategies in Uruguay. One week after weaning, 96 purebred V line rabbits were randomly distributed between 2 treatments: (T1) commercial pelleted food *ad libitum* and (T2) commercial pelleted food *ad libitum* plus fresh alfalfa *ad libitum*. Each treatment included 12 cages containing 4 individuals each (2 males and 2 females). Growth performance characteristics (live weight evolution, commercial food consumption and food/gain ratio) were evaluated. The consumption of alfalfa was not measured. Rabbits were slaughtered at a live weight of 2500 g and carcass characteristics were evaluated. Samples of meat and dissectible fat were analysed to determine intramuscular fat content at muscle *L. dorsi*, dissectible fat and intramuscular fat composition, minerals (Zn, Fe, Mg and Na), vitamin E and purines. Sensory evaluations were conducted to assess the effect of treatments on the consumer's perception of differences and the existence of attributes determining preferences. Differences between treatments were significant for total commercial food intake (23356 vs. 20930 g/cage;  $P<0.001$ ) and feed conversion ratio (3.82 vs. 3.41;  $P<0.01$ ) for T1 and T2 respectively. No significant differences were found in average daily gain, age at slaughter and carcass characteristics. There were no significant differences in the intramuscular fat content. The fatty acid composition of dissectible and intramuscular fat was affected by the inclusion of alfalfa in the diet increasing the linolenic acid content (1.82 vs. 3.28% and 2.29 vs. 5.15% for T1 and T2 at intramuscular and dissectible fat, respectively;  $P<0.001$ ), and improving the n-6/n-3 relationship (8.60 vs. 5.82 and 11.58 vs. 5.64 for T1 and T2 at intramuscular and dissectible fat, respectively;  $P<0.001$ ). There were no significant differences in vitamin E, Fe or Zn content between treatments, but differences were significant in Mg (22.5 vs. 24.4 mg/100 g for T1 and T2;  $P<0.05$ ) and Na (44.1 vs. 48.2 mg/100 g;  $P<0.05$ ). In the sensory evaluation, panellists significantly perceived differences between treatments with 95% confidence.

**Key Words:** rabbit meat, fat composition, purines, minerals, vitamin E.

## INTRODUCTION

The use of fresh forage as a partial substitute for commercial pelleted food is a traditional feeding strategy in meat rabbit production in Uruguay. The aim is to reduce the production costs and the incidence of uncontrolled variations in the price of feed, the most important component of the production costs structure. An investigation conducted in the late 90s to characterise rabbit production systems in the country stated that 73% of rabbit breeders used exclusively commercial feed and only 27% combined the feed with fresh forage (Pérez and Velázquez, 1998). A more recent study verified the use of fresh forage by 20% of the producers (Amoza *et al.*, 2008).

There is information generated by national research about the effect of the inclusion of different fresh forages in the diet on the production performance of growing rabbits (Blumetto and Capra, 1998a) and lactating does (Blumetto and

**Correspondence:** G. Capra, gcapra@ib.inia.org.uy Received March 2012 - Accepted October 2012.

<http://dx.doi.org/10.4995/wrs.2013.1197>

Capra, 1998b). The results highlighted the effectiveness of alfalfa for this purpose, enhanced for its productivity in the southern region, which allows high quality forage at a very low cost.

The effect of diet composition on the lipid profile of rabbit meat has been confirmed by numerous authors (Dalle Zotte, 2000; Hernández, 2008; Webb and O'Neill, 2008; Hernández and Dalle Zotte, 2010). This attribute has led to the search for modifications in the diet that effectively contribute to the enrichment of the nutritional value of rabbit meat. Zhang *et al.* (2010) in a thorough review describe the improvement of the functional value of meat by dietary supplementation with ingredients that enhance the contribution of bioactive compounds such as conjugated linoleic acid (CLA), vitamin E, n-3 fatty acids and selenium. Hernández (2012) affirms that rabbit meat can be a good way to provide consumers with healthy compounds and emphasises its potential as a functional food. Dalle Zotte and Szendrő (2010) point out that management of rabbit diet composition has been very effective in increasing levels of essential fatty acids, EPA, DHA, CLA, branched chain fatty acids, vitamin E and selenium in the meat. Many research papers have focused the objective on increasing the content of the rabbit meat in n-3 polyunsaturated fatty acids and improving the n-6/n-3 ratio (Oliver *et al.*, 1997; Gigaud and Le Cren, 2006; Maertens *et al.*, 2008; Tres *et al.*, 2008; Kowalska and Bielanski, 2009; Petracci *et al.*, 2009).

Alfalfa is a very important linolenic acid (C18:3 n-3) source and the positive effect of including dehydrated alfalfa on the lipid profile and the n-6/n-3 ratio has been demonstrated (Combes and Cauquil, 2006). Capra *et al.* (2010) showed a significant increase in linolenic acid content in the intramuscular and dissectible fat when including fresh alfalfa *ad libitum* in the diet of growing rabbits in Uruguay.

The use of fresh alfalfa in the diet of meat rabbits is, in Uruguayan conditions, a particularly suitable strategy for small farmers. The confirmation of a positive effect on the rabbit meat nutritive value obtained with this strategy can help to stimulate domestic consumption, differentiating and enhancing the product quality and improving the economic performance of small-scale rabbit breeders. The objectives of this study focus on the evaluation of the effect of including alfalfa *ad libitum* in the diet on the main production performance parameters, carcass characteristics, nutritional value and sensory properties of rabbit meat. From the nutritional standpoint, the study focuses on intramuscular fat content, intramuscular and dissectible fat composition, vitamin E, 4 selected minerals (Fe, Zn, Na and Mg) and purine content. Purine content was included in this study with the purpose of assessing the potential use of rabbit meat in diets for hyperuricemics, taking into account the existence of contradictory information about purine content in rabbit meat.

## MATERIALS AND METHODS

### *Animals and feed*

One week after weaning, 96 purebred V line rabbits, weaned at 30 d of age, were randomly distributed between 2 feeding treatments: (T1) commercial pelleted food *ad libitum* and (T2) commercial pelleted food *ad libitum* plus fresh alfalfa *ad libitum*. The experiment was conducted at the Rabbit Experimental Unit of INIA in Uruguay, at the "Wilson Ferreira Aldunate" Experimental Station, Rincón del Colorado, Canelones, between February 2<sup>nd</sup> and March 29<sup>th</sup>, 2010. The rabbits were born between December 18<sup>th</sup> and 24<sup>th</sup>, 2009 and at the beginning of the experiment had an average weight of 964 g. Each treatment consisted of 12 cages with 4 rabbits each (2 male and 2 female). Galvanised wire cages 0.86×0.40×0.33 m (length× width×height) were used for the growing-fattening period. The cages had a hopper feeder and an automatic cup drinker and were located in an open-air shelter with asphalted cardboard roofing.

The feed ingredients and chemical composition of the commercial pelleted food are shown in Table 1. The weight of the pelleted food supplied was recorded each day and once a week the rejection was weighed to obtain the weekly consumption per cage by difference.

Alfalfa from a first year crop was in the flowering onset stage at the start of the trial. It was cut daily and placed on the cage roof in amounts that ensured the rabbits always had food at will. Each week samples were drawn for determination of dry matter, which averaged 20.1% throughout the period. Although the weight of alfalfa offered was recorded daily, the real consumption was not determined.

**Table 1:** Ingredients and chemical composition of the commercial pelleted feed.

Ingredients (kg per metric ton)	
Alfalfa hay	360.0
Wheat bran	120.0
Corn	111.7
Sunflower meal	100.0
Wheat middlings	80.0
Soybean meal	70.0
Wheat flour	70.0
Oats	60.0
Dicalcium phosphate	13.1
Calcium carbonate	5.6
Salt	5.4
Vitamin-mineral premix VM-602	2.0
Calcium propionate	1.0
Zinc bacitracin	0.5
DL-methionine 99%	0.4
L-Lysine 95%	0.3
Chemical composition (% on dry basis)	
Dry matter (%)	86.8
Crude Protein	21.3
Acid Detergent Fibre	27.0
Neutral Detergent Fibre	38.0
Ether Extract	3.6
Calcium	1.9
Phosphorus	1.1

Table 2 shows the lipid profile of both feeds used in this study. In the commercial pelleted food, the fatty acids sum of each group was: saturated fatty acids (SFA): 16.5%, monounsaturated fatty acids (MUFA): 26.6%, polyunsaturated fatty acids (PUFA): 55.3%, with an n6/n3 ratio of 8.22.

The rabbits were individually weighed weekly. When the usual Uruguayan slaughter weight was reached (about 2500 g), all 96 rabbits were slaughtered in the experimental farm, without being subject to previous fasting. This determined 6 different slaughter days, between 9<sup>th</sup>-24<sup>th</sup> March, 2010. The average slaughter weight for all animals in the trial was 2506±84 g. Although the choice of slaughtering rabbits at a fixed weight may be questionable, this criteria was adopted taking into account the practical implications for rabbit farmers, as usual market weight standards are very narrow in Uruguay (rabbits slaughter weight must range 2400-2700 g, otherwise price is penalised).

### *Carcass evaluation.*

The evaluation included all 96 rabbit carcasses and took place at the Laboratorio Tecnológico del Uruguay (LATU). The harmonised procedure described by Blasco and Ouhayoun (1996) and extended by Ouhayoun and Dalle Zotte (1996) and Pla and Dalle Zotte (2000), was followed. The weight of the so-called "Uruguayan carcass" (UCW) was included, corresponding to the headless carcass, with liver and kidneys, which is the usual way of marketing meat rabbits in Uruguay. The carcass yield expressed in percentage of live weight (LVW) at slaughter was also calculated (%UCW/LVW). Deboning took place 24 h after slaughter, after cooling the carcass in a 4°C chamber. The pH was also measured after 24 h in the *L. dorsi* muscle between the 4<sup>th</sup> and the 5<sup>th</sup> lumbar vertebra, using a pH-meter with automatic temperature compensation (Seven multi, Mettler Toledo, Switzerland).

**Table 2:** Lipid content and fatty acid composition of feeds (%).

	Commercial food	Alfalfa
Total lipids (%)	3.35	0.19
Total identified fatty acids (%)	98.4	99.7
C14:0	0.3	-
C16:0	12.5	12.6
C16:1 n-7	0.9	1.4
C18:0	2.7	1.7
C18:1 n-9	23.9	1.8
C18:2 n-6	49.3	13.1
C18:3 n-6	0.8	-
C18:3 n-3	5.2	65.7
C20:0	0.5	-
C20:1 n-9	0.3	1.3
C22:0	0.5	-
C22:1	1.5	2.1
∑SFA	16.5	14.3
∑MUFA	26.6	4.5
∑PUFA	55.3	78.8

### Analytical determinations

The intramuscular fat content, fat lipid profile, mineral and vitamin E content and sensory analyses were performed at LATU, while the purines content was analysed at INIA.

**Lipid profile.** To determine the intramuscular fat lipid profile, 20 samples from muscle *L. dorsi* of 30 g each were used, 10 from each feeding treatment. For the dissectible fat lipid profile analysis, 20 samples were randomly selected, 6 from each feeding treatment. The dissectible fat included perirenal, scapular and inguinal fat. The samples were crushed and homogenised in a domestic processor prior to their vacuum packaging (SuperVac GK269/G, Austria) in multilayer film bags with low permeability to oxygen and water vapour (Perflex Clear-Tite 62) and frozen at  $-18^{\circ}\text{C}$  until analysis. The fatty acid content was determined by gas chromatography and mass spectrometry following the AOCS Official Method Ce 2-66 "Preparation of Methyl Esters of Fatty Acids", AOCS Ce 1-62 "Fatty Acid Composition by Gas Chromatography", AOCS Ce 1-91 "Preparation of Methyl Esters of Long Chain Fatty Acids".

**Minerals.** Twenty samples of 5 g each were used for the Fe, Na, Zn and Mg analysis, 10 from each feeding treatment. The samples were taken from a meat pool obtained by crushing and homogenising the whole carcass meat with a domestic processor. The samples were vacuum packaged as before and frozen at  $-18^{\circ}\text{C}$  until their analysis. Fe, Na, Zn and Mg were analysed in digested samples in a closed high pressure system following the AOAC 999.10 adapted method, by atomic emission (ICP-OES) based on adapted ISO 11885:1996.

**Vitamin E.** For the vitamin E analysis 20 samples of 10 g each one were used, 10 from each feeding treatment. Samples for these analyses were obtained from the whole carcass meat pool and processed in the same way as those for the lipid profile analysis. They were vacuum packaged as described above, frozen and foil covered to avoid light exposure. The vitamin E was determined following EN 12822, consisting of saponification and extraction in organic solvent. The extracted organic layer was injected directly into high pressure liquid chromatography (HPLC) after filtering through  $0.22\ \mu\text{m}$ . Chromatographic separation was performed on normal phase (Phenomenex Silica) using a fluorescence detector at 290-330 nm.

**Purines.** Ten samples of raw meat from each feeding treatment were used for the purines analysis. These samples were processed, vacuum packaged and frozen in the same way as the Vitamin E samples. For the purine content analysis of the meat, a hydrolysis of the sample was made in acid conditions (perchloric acid 2 M). The hydrolysed

was neutralised and the uricogenic potential purines (hypoxanthine, guanine, xanthine and adenine) were analysed. The analysis was performed by HPLC with a UV detector (254 nm) and a reverse phase column RP-C18 (Vani *et al.*, 2006; Fan *et al.*, 2007; Reynal and Broderick, 2009). Calibration curves were made for each purine with  $R^2 > 0.99$ .

### Sensory analysis

For the preference, texture and overall liking analysis, 62 samples of rabbit loins from each feeding treatment were vacuum packaged as for analytical determinations and frozen at  $-18^\circ\text{C}$  until their analysis.

The triangular test with consumers was the method employed for the preference study between 2 samples. The samples were pan-cooked until the centre temperature reached  $70^\circ\text{C}$ . They were served to the consumers hot and cut into prisms of  $3 \times 1.5 \times 1.5$  cm on three-digit random-number coded transparent plates randomly arranged on the display tray. The analysis was carried out with a panel of 40 consumers where 40% were female, in a standard room according to ISO 8589:1988, under white artificial light and controlled temperature ranging  $22\text{--}24^\circ\text{C}$ . The results were statistically evaluated with a 95% confidence level.

For the sensory evaluation of texture and overall liking of the samples, a structured hedonic scale of 9 points was used (1- dislike extremely, 5- neither like nor dislike, 9- like extremely). An analysis of variance for each attribute was performed and the minimum significant difference was calculated using the Tukey test ( $P < 0.05$ ) with Infostat 2008. The assessment was performed with a panel of 38 consumers where 45% were female.

### Statistical analysis

The experimental unit for the consumption and the conversion rate was the 4 rabbit cage. For the other evaluated parameters, the experimental unit was each rabbit. The results were analysed using the GLM procedure of SAS 2003, with the treatment and gender included in the model as fixed effects for growth performance and carcass parameters. For the growth parameters, the initial weight was included as covariate and for carcass evaluation parameters the average daily weight gain was included as covariate.

## RESULTS AND DISCUSSION

Table 3 shows the growing-fattening parameters obtained. The inclusion of alfalfa *ad libitum* in the T2 treatment determined a significant drop in the commercial pelleted food consumption, around 11%, without producing a negative effect on growth rate and age at slaughter. These results are similar to those obtained previously (Capra *et al.*, 2010) with crossbred R×V rabbits that yielded a mean reduction of 12.6% in the pelleted food consumption. In the cited experiment, the differences between treatments at slaughter age were significant (75.4 vs. 78.7 d for without alfalfa and with alfalfa respectively;  $P < 0.05$ ), a fact not found in this case. Differences in variability in slaughter age between these 2 experiments may be attributed to genetic type.

Table 4 shows the results of the carcass characteristics evaluation. No statistically significant differences were found for any of the parameters assessed. These results contrast with those obtained with crossbred RxV rabbits on identical diets, in which significant differences were observed in yield of reference carcass, percentage of meat in the reference carcass and meat/bone ratio (Capra *et al.*, 2010). The dissectible fat values obtained both in absolute terms and in

**Table 3:** Effect of feeding strategy on rabbit growth performance (mean±standard deviation).

Variable	Without alfalfa	With alfalfa	P-value
Initial weight (g)	976±120	952±93	NS
Slaughter weight (g)	2509±77	2511±64	NS
Total commercial feed consumption (g/cage)	23536±1210	20930±1960	$P < 0.0001$
Feed conversion ratio	3.82±0.32	3.41±0.24	$P < 0.01$
Age at slaughter (d)	88.7±7.1	90.7±5.9	NS
Average daily gain (g/d)	34.7±5.6	32.9±4.2	NS

NS: no significant.

**Table 4:** Effect of feeding strategy on carcass characteristics (mean±standard deviation).

Variable	Without alfalfa	With alfalfa	P-value
Intramuscular fat g/100g	1.41±0.34	1.39±0.33	NS
pH 24 h	5.57±0.11	5.59±0.11	NS
UCW (g)	1369±56	1366±53	NS
% UCW/LVW	54.6±1.0	54.4±1.9	NS
RCW (g)	1265±52	1264±54	NS
% RCW/LVW	50.6±1.4	50.3±2.1	NS
% DFaW/RCW	2.49±0.68	2.40±0.57	NS
% MW/RCW	75.9±0.9	76.0±0.9	NS
% BW/RCW	20.7±1.0	20.8±0.8	NS
M/B	3.63±0.34	3.65±0.16	NS
M/B HL	4.93±0.43	5.08±0.35	NS

UCW: Uruguayan carcass weight. % UCW/LVW: Uruguayan carcass yield expressed as percentage of live weight at slaughter. RCW: Reference carcass weight. % RCW/LVW: Reference carcass yield expressed as percentage of live weight at slaughter. % DFaW/RCW: Percentage of dissectible fat of the RCW. % MW/RCW: Meat yield expressed in percentage of the RCW. % BW/RCW: Bone percentage of RCW. M/B: Meat total weight to bone total weight ratio of the carcass. M/B HL: Meat to bone ratio on the hind leg. NS: no significant.

its relative value to the reference carcass weight are very similar to those achieved in the experiment mentioned. The values obtained for these 2 parameters are below those ones achieved by Pla *et al.* (1998) for Verde line rabbits slaughtered at lower weights than the usual slaughter weights for Uruguay. In contrast, the meat to bone ratio on the hind leg achieved in this experiment is above the ratio obtained by the cited authors.

Sex had no statistically significant effects on the various parameters of production performance and carcass characteristics under evaluation. These results agree with those reported by Ortiz Hernandez and Rubio Lozano (2001) and Yalçin *et al.* (2006).

Table 5 shows the treatment effect on the dissectible and intramuscular fat composition and on different fatty acid ratios that are important from the standpoint of the effect of fat on consumer health. These indicators include the n-6/n-3 ratio, the atherogenicity (AI) and thrombogenicity (TI) indexes proposed by Ulbricht y Southgate (1991) and the ratio h/H between the sum of hypocholesterolemic fatty acids (monounsaturated and polyunsaturated) and the hypercholesterolemic (miristic and palmitic) as defined by Herranz *et al.* (2008).

The fatty acids in greater proportion are palmitic C16:0, oleic C18:1 and linoleic C18:2 n-6, in similar values to those reported by Dalle Zotte (2002), Combes and Dalle Zotte (2005), Combes and Cauquil (2006), Hernández (2008), Kowalska and Bielanski (2009). However, they are substantially different from the values reported by Ramírez (2004) who verified a 31.4% content of linoleic acid, and by Lazzaroni *et al.* (2009) who determined 14.3% of the same fatty acid. Although there are several factors affecting the fat composition (sex, housing system, genetic type, age, slaughter weight), in monogastrics the diet has a decisive relevance (Dalle Zotte, 2000; Pla, 2004). Gigaud and Le Cren (2006) state that the nutritive value of rabbit meat is strongly correlated with the fatty acid profile of the animal diet's raw materials.

The incorporation of fresh alfalfa *ad libitum* in the diet of growing rabbits showed a significant increase in the linolenic acid C18:3 n-3 in both intramuscular and dissectible fat. These results agree with those obtained before in similar experiments (Capra *et al.*, 2010).

It is remarkable that measurable levels of long chain n-3 PUFA eicosapentaenoic (EPA) C20:5, docosapentaenoic (DPA) C22:5 and docosahexaenoic (DHA) C22:6 were identified in the intramuscular fat, but not in the dissectible fat. The inclusion of alfalfa in the diet results in a significant increase of DPA levels. It is evident that the content of these n-3 fatty acids is relatively low and with great variability. Combes and Cauquil (2006) affirm that there is individual variability in the ability to deposit linolenic acid in the meat. It is also suggested that an increase in the linolenic acid (n-3 PUFA precursor) input stimulates biosynthesis by elongation and desaturation of linolenic (Combes and Cauquil, 2006; Tres *et al.*, 2008).

**Table 5:** Effect of feeding strategy on the dissectible and intramuscular fat composition (%; mean±standard deviation).

Fatty acid	Intramuscular fat			Dissectible fat		
	Without alfalfa	With alfalfa	P-value	Without alfalfa	With alfalfa	P-value
C12:0	0.07±0.01	0.07±0.02	NS	0.25±0.17	0.18±0.14	NS
C14:0	1.88±0.27	1.58±0.33	P<0.05	2.36±0.35	2.13±0.26	NS
C16:0	28.66±1.89	28.04±1.56	NS	31.49±2.86	29.65±0.71	NS
C18:0	8.44±0.74	8.85±0.74	NS	5.64±0.72	6.22±0.56	NS
C16:1cis	4.13 ±1.19	2.95±0.80	P<0.05	2.34±0.54	1.35±0.32	P<0.01
C18:1cis	27.50±1.67	24.70±2.46	P<0.01	27.27±1.47	25.13±1.37	P<0.05
C18:2 (n-6)	21.27±1.84	22.24±2.63	NS	26.58±3.80	28.70±2.04	NS
C18:3 (n-3)	1.82±0.50	3.28±0.63	P<0.001	2.29±0.45	5.15±0.68	P<0.001
C20:4 (n-6)	2.18±0.97	3.45±1.47	P<0.05	0.07±0.01	0.08±0.01	NS
C20:5 (n-3)	0.25±0.19	0.18±0.11	NS	-	-	-
C22:5 (n-3)	0.12±0.10	0.47±0.25	P<0.05	-	-	-
C22:6 (n-3)	0.20±0.12	0.37±0.34	NS	-	-	-
ΣSFA	40.36±2.37	39.91±1.99	NS	40.76±3.12	39.37±0.81	NS
ΣMUFA	32.10±2.47	28.30±2.57	P<0.01	30.10±2.10	26.73±1.58	P<0.05
ΣPUFA	27.04±3.51	31.15±4.00	NS	29.02±3.91	33.89±2.04	P<0.05
PUFA/SFA	0.68±0.12	0.79±0.13	NS	0.72±0.15	0.86±0.06	NS
SFA/(MUFA+PUFA)	0.69±0.07	0.67±0.06	NS	0.69±0.09	0.65±0.02	NS
Σ(n-6)	23.59±2.79	25.83±3.55	NS	26.77±3.72	28.74±2.00	NS
Σ(n-3)	2.81±0.67	4.55±0.81	P<0.001	2.38±0.48	5.18±0.71	P<0.001
n-6/n-3	8.60±1.21	5.82±1.19	P<0.001	11.58±2.34	5.64±0.86	P<0.001
AI <sup>1</sup>	0.62±0.07	0.58±0.06	NS	0.70±0.10	0.63±0.03	NS
TI <sup>2</sup>	0.96±0.12	0.80±0.10	P<0.01	1.12±0.17	0.88±0.04	P<0.01
h/H <sup>3</sup>	1.95±0.21	2.02±0.17	NS	1.77±0.21	1.91±0.06	NS

<sup>1</sup> Atherogenicity Index: AI=[C12:0+(4×C14:0)+C16:0]/[(ΣPUFA)+(ΣMUFA)]. <sup>2</sup> Thrombogenicity Index: TI=[C14:0+C16:0+C18:0]/[(0.5×ΣMUFA)+(0.5×Σn-6)+(3×Σn-3)+(n-3/n-6)]. <sup>3</sup> h/H=(ΣMUFA+ΣPUFA)/(C14:0+C16:0). NS: no significant.

The fatty acid composition modified by the alfalfa inclusion in the diet has a propitious effect in all the indexes used to evaluate the nutritive value and the potential impact on consumer health. One of the most used indicators, the n-6/n-3 ratio, has relevant differences between both treatments.

The AI and TI values proposed by Ulbricht and Southgate (1991) also showed an improvement with the alfalfa inclusion, particularly on the dissectible fat. Both indexes consider the effect of the different fatty acids related with the incidence of coronary diseases.

The values obtained in this experiment showed significant differences in TI between treatments, with a positive effect of the alfalfa inclusion. The AI had lower values and the TI slightly higher values that those reported by Lazzaroni *et al.* (2009) in rabbits raised in 2 different housing systems.

The h/H ratio shown in Table 5 is another index used to estimate the nutritive attributes of food (Herranz *et al.*, 2008). In this experiment, no statistically significant differences were observed between treatments in this ratio, either in intramuscular or dissectible fat.

The feeding strategy including fresh alfalfa significantly improves the composition of fat, mainly by the increase in linolenic acid C18:3 n-3. This results in a better balance in the ratio n6/n3, considered an important factor for preventing cardiovascular disease and cancer (Carrero *et al.*, 2005; Béliveau and Gingras, 2007). According to López-Farré and Macaya (2006), 3 main mechanisms are involved in the cardiovascular protective effect of n-3 fatty acids: their anti-inflammatory effect, antithrombotic effect and anti-arrhythmic action.

Table 6 presents the comparison of the treatments for the content of some minerals (Na, Fe, Mg, and Zn) and vitamin E.

The values obtained for Na and Mg are framed within those reported in the literature (Dalle Zotte, 2002; Combes and Dalle Zotte, 2005; Gigaud and Le Cren, 2006; Hermida *et al.*, 2006), while the Fe level is below and the Zn level over

**Table 6:** Effect of feeding strategy on minerals and vitamin E contents (mean±standard deviation).

	Without alfalfa	With alfalfa	P-value
Na (mg/100 g)	44.10±0.49	48.20±0.30	P<0.05
Fe (mg/100 g)	0.63±0.46	0.65±0.66	NS
Mg (mg/100 g)	22.50±0.17	24.40±0.16	P<0.05
Zn (mg/100 g)	1.29±0.11	1.34±0.14	NS
Vitamin E (mg $\alpha$ -tocoferol/100 g)	0.27±0.04	0.31±0.05	NS

NS: no significant.

the range reported by these authors. Hermida *et al.* (2006) affirm that the low Na and high K contents make the rabbit meat particularly recommended in diets for hypertension. These authors also remark that rabbit meat provides less Zn and Fe than meat from other species.

In the case of vitamin E, the values obtained in this experiment are greater than the 0.186 mg/100 g reported by Combes and Dalle Zotte (2005) and similar to the range of 0.181-0.376 mg/100 g obtained for different feeding treatments by Kowalska and Bielanski (2009).

Purines content determined in rabbit meat for both treatments is presented in Table 7. As was presumed, there were no significant differences between treatments in purine contents, since species, processing and cooking method are the main factors that determine purine levels in meat.

The information available about purines content in rabbit meat is very limited, aggravated by differences in the methodology for its determination and pre-processing of the meat.

Rabbit meat has been promoted for its low purines content which would make it particularly recommended in diets for hyperuricemia (Bixquert and Gil-Borrás, 2005; Hernández, 2008). However, other sources such as Souci-Fachmann-Kraut tables of nutrient composition of foods located rabbit meat among the foods with a moderate level of purines ranging from 95-150 mg uric acid/100 g.

Brulé *et al.* (1988) argue that purines have a different metabolic effect and assert that adenine and hypoxanthine consumption modifies the serum and urinary uric acid level, while guanine and xanthine do not show this effect. These authors reported that while there is wide variation in the total content of purines in foods, virtually all meat products have similar values for the sum of adenine+hypoxanthine, in the range of 100 to 150 mg/100 g of food. It should be noted that the purine contents reported by these authors for common Canadian foods are not comparable with those obtained in this work, since the foods were subjected to cooking treatment that modifies the purines content.

The sensory evaluation performed with a consumer panel at the Laboratorio Tecnológico del Uruguay showed that consumers are able to differentiate meat from both treatments with a 95 % confidence interval. However the sensory evaluation of texture attributes and overall liking of the samples showed no statistically significant differences (Table 8). These results suggest that the inclusion of fresh alfalfa *ad libitum* in the rabbit's diet imprints a change in the sensory qualities of rabbit meat which allow consumers to distinguish it from the meat of rabbits fed only with commercial pelleted food, but this effect does not make a significant change in its overall liking.

**Table 7:** Effect of feeding strategy on purines content of raw rabbit meat mg/100 g (mean±standard deviation).

	Without alfalfa	With alfalfa	P-value
Guanine	20.7±2.6	19.7±1.9	NS
Hypoxanthine	82.1±7.4	80.6±6.4	NS
Xanthine	n.d.	n.d <sup>1</sup>	-
Adenine	23.6±2.8	21.8±2.0	NS

<sup>1</sup>n.d.: no detected.

NS: no significant.

**Table 8:** Results of sensory evaluation of texture and overall liking.

	Without alfalfa	With alfalfa	Significance
Texture	6.8	6.5	NS
Overall liking	6.9	6.6	NS

NS: no significant.

## CONCLUSIONS

The inclusion of fresh alfalfa *ad libitum* in diet for growing rabbits should be considered as an alternative in the design of feeding strategies for growing-finishing meat rabbits. Under the Uruguayan production conditions, the partial substitution of commercial feed with fresh forage means a reduction in production costs, which is not detrimental to the economic characteristics of the carcass.

From the point of view of the nutritional attributes of meat, the inclusion of fresh alfalfa determines a favourable change in fat composition, with a significant increase in the content of linolenic acid C18:1 n-3. The quantitative increase of n-3 fatty acids may be considered a modest improvement from the nutritional point of view. Nevertheless, a 200 g serving of rabbit loin of T2 accounts for 7% of n-3 nutritional goals (MSP, 2005). The n-3 increase is reflected in a significant improvement in n-6/n-3 ratio and other indicators of nutritive value and impact on consumer health. Other changes in the composition of rabbit meat, such as the slight increase in the contribution of Na and Mg, or the tendency to improve the supply of vitamin E, are negligible from the nutritional standpoint because of their low magnitude.

The average content of purines in rabbit meat verified in this experiment approximates the values of other meats within the group of foods with moderate purine content.

The inclusion of fresh alfalfa does not determine significant effects on texture and overall liking of meat, although its sensory attributes are perceived as different from the meat of rabbits fed exclusively on commercial pelleted food.

**Acknowledgements:** Phytochemical Laboratory, INIA Las Brujas. Rabbit Experimental Unit, INIA. Department of Analytical Methods Development, LATU. Department of Dairy, Meat, Fruit, Vegetable and Beehive products, LATU. Department of Atomic Spectrometry for Food and Environment, LATU. The authors thank Dr. Gabriel Ciappesoni for his cooperation in the statistical analysis and Dr. Pilar Hernández for her invaluable suggestions.

## REFERENCES

- Amoza C., Lusardo R., Oliver L. 2008. La explotación de la cunicultura y su fomento en el Uruguay. *Facultad de Ciencias Económicas y Administración. Universidad de la República. 170 pp.*
- Béliveau R., Gingras D. 2007. Los alimentos contra el cáncer. La prevención del cáncer a través de la alimentación. *El Ateneo, Buenos Aires, Argentina.*
- Bixquert Jiménez M., Gil-Borrás R. 2005. Propiedades nutricionales y digestibilidad de la carne de conejo. *Revista Científica de Nutrición, 1. Available at: <http://www.intercun.org>. Accessed January 2012.*
- 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*
- Blumetto O., Capra G. 1998a. Utilización estratégica de forrajes en la alimentación de conejos. *Serie Hojas de Divulgación, INIA, Montevideo, Uruguay, 68.*
- Blumetto O., Capra G. 1998b. Suplementación con alfalfa fresca a conejas en lactación. *Serie Hojas de Divulgación, INIA, Montevideo, Uruguay, 69.*
- Brulé D., Sarwar G., Savoiet L. 1988. Purine content of selected Canadian food products. *J. Food Compos. Anal., 1: 130-138. doi:10.1016/0889-1575(88)90016-6*
- Capra G., Grompone M.A., Urruzola N., Pardo M.J., Martínez R., Fradiletti F., Cozzano S., Repiso L., Márquez R. 2010. Effect of fresh alfalfa in the diet of growing rabbits on growth performance, carcass characteristics and fat composition. *In Proc.: IV Congreso Cunicultura de las Américas, 22-24 September, 2010. Córdoba, Argentina.*
- Carrero J.J., Martín-Bautista E., Baró L., Fonollá J., Jiménez J. Boza J.J., López-Huertas E. 2005. Efectos cardiovasculares de los ácidos grasos omega-3 y alternativas para incrementar su ingesta. *Nutr. Hosp., 20: 63-69.*
- Combes S., Cauquil L. 2006. Une alimentation riche en luzerne permet d'enrichir la viande des lapins en oméga 3. *Viandes et Produits Carnés., 25: 31-35.*
- Combes S., Dalle Zotte A. 2005. Le viande de lapin: valeur nutritionnelle et particularités technologiques. *In Proc.: 11<sup>èmes</sup>. Journées de la Recherche Cunicole, 29-30 November, 2005. Paris, France, 167-180.*

- Dalle Zotte A. 2000. Main factors influencing the rabbit carcass and meat quality. In *Proc.: 7<sup>th</sup> World Rabbit Congress*, 4-7 July, 2000. Valencia, Spain. *World Rabbit Sci.*, 8, Suppl.1: 507-537.
- Dalle Zotte A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livest. Prod. Sci.*, 75: 11-32. doi:10.1016/S0301-6226(01)00308-6
- Dalle Zotte A., Szendrői Zs. 2010. The role of rabbit meat as functional food. In *Proc.: IV Congreso de Cunicultura de las Américas*, 22-24 September, 2010. Córdoba, Argentina, 30 pp.
- Fan H., Yang F.Q., Li S.P. 2007. Determination of purine and pyrimidine bases in natural and cultured *Cordyceps* using optimum acid hydrolysis followed by high performance liquid chromatography. *J. Pharmaceut. Biomed.*, 45: 141-144. doi:10.1016/j.jpba.2007.02.032
- Gigaud V., Le Cren D. 2006. Valeur nutritionnelle de la viande de lapin et influence du régime alimentaire sur la composition en acides gras. *Journée nationale ITAVI Élevage du Lapin de Chair*, Pacé, Nov. 2006, 45-57.
- Hermida M., González M., Miranda M., Rodríguez-Otero J.L. 2006. Mineral analysis in rabbit meat from Galicia (NW Spain). *Meat Sci.*, 73: 635-639. doi:10.1016/j.meatsci.2006.03.004
- Herranz B., Ordóñez J.A., De La Hoz L., Hierro E., Soto E., Cambero M.I. 2008. Fatty acid composition of salami from different countries and their nutritional implications. *Int. J. Food Sci. Nutr.*, 59: 607-618. doi:10.1080/09513590701550270
- Hernández P. 2008. Enhancement of nutritional quality and safety in rabbit meat. In *Proc.: 9<sup>th</sup> World Rabbit Congress*. 10-13 June, 2008. Verona, Italy, 1287-1299.
- Hernández P. 2012. Carne de conejo como alimento funcional. *Cunicultura*, 37: 21-24.
- Hernández P., Dalle Zotte A. 2010. Influence of the Diet on Rabbit Meat Quality. In: *Nutrition of the Rabbit*, 2<sup>nd</sup> edition. C. de Blas, J. Wiseman (ed.). CABI, UK, 163-178.
- Kowalska D., Bielanski P. 2009. Meat quality of rabbits fed a diet supplemented with fish oil and antioxidant. *Anim. Sci. Pap. Rep.*, 27: 139-148.
- Lazzaroni C., Biagini D., Lussiana C. 2009. Fatty acid composition of meat and perirenal fat in rabbits from two different rearing systems. *Meat Sci.*, 83: 135-139 doi:10.1016/j.meatsci.2009.04.011
- López-Farré A., Macaya C. 2006. Efectos antitrombóticos y antiinflamatorios de los ácidos grasos omega-3. *Rev. Esp. Cardiol. Supl.*, 6: 31D-37D. doi:10.1157/13091597
- Maertens L., Huyghebaert G., Delezie E. 2008. Fatty acid composition of rabbit meat when fed a linseed based diet during different periods after weaning. In *Proc.: 9<sup>th</sup> World Rabbit Congress*, 10-13 June, 2008. Verona, Italy, 1381-1384.
- Ministerio de Salud Pública, Dirección General de la Salud, Uruguay. 2005. Manual para la promoción de prácticas saludables de alimentación en la población uruguaya. Montevideo, Uruguay: MSP. 115 pp. Available at: [http://www.msp.gub.uy/uc\\_123\\_1.html](http://www.msp.gub.uy/uc_123_1.html) Accessed January 2012
- Oliver M.A., Guerrero L., Díaz I., Gispert M., Pla M., Blasco A. 1997. The effect of fat-enriched diets on the perirenal fat quality and sensory characteristics of meat from rabbits. *Meat Sci.*, 47: 95-103. doi:10.1016/S0309-1740(97)00041-7
- Ortiz Hernández J.A., Rubio Lozano M.S. 2001. Effect of breed and sex on rabbit carcass yield and meat quality. *World Rabbit Sci.*, 9: 51-56 doi:10.4995/wrs.2001.445
- Ouhayoun J., Dalle Zotte A. 1996. Harmonization of Muscle and Meat Criteria in Rabbit Meat Research. *World Rabbit Sci.*, 4:211-218. doi:10.4995/wrs.1996.297
- Pérez M., Velázquez D. 1998. Evaluación de la mortalidad perinatal de los gazapos y sus posibles causas. Diagnóstico a nivel nacional. *Facultad de Agronomía, Universidad de la República*, 30 pp.
- Petracci M., Bianchi M., Cavani C. 2009. Development of rabbit meat products fortified with n-3 Polyunsaturated Fatty Acids. *Nutrients*, 1: 111-118. doi:10.3390/nu1020111
- Pla M. 2004. Effects of nutrition and selection on meat quality. In *Proc.: 8<sup>th</sup> World Rabbit Congress*. 7-10 September, 2004. Puebla, México, 1337-1348.
- Pla M., Dalle Zotte A. 2000. Harmonisation of criteria and methods used in rabbit meat research. In *Proc.: 7<sup>th</sup> World Rabbit Congress*, 4-7 July, 2000. Valencia, Spain. *World Rabbit Sci.*, 8, Suppl.1: 539-545.
- Pla M., Guerrero L., Guardia D., Oliver M.A., Blasco A. 1998. Carcass characteristics and meat quality of rabbits lines selected for different objectives: I. Between lines comparison. *Livest. Prod. Sci.*, 54: 115-123. doi:10.1016/S0301-6226(97)00179-6
- Ramírez J.A. 2004. Características bioquímicas del músculo, calidad de la carne y de la grasa de conejos seleccionados por velocidad de crecimiento. *Tesis Doctoral. Universidad Autónoma de Barcelona, España*. 180 pp.
- Reynal S.M., Broderick G.A. 2009. A new high-performance liquid chromatography purine assay for quantifying microbial flow. *J. Dairy Sci.*, 92: 1177-1181. doi:10.3168/jds.2008-1479
- SAS. 2003. SAS version 9.1.3. *SAS Inst., Cary N.C., USA*.
- Souci-Fachmann-Kraut's. Food Composition Nutritional Tables, 6<sup>th</sup> ed. Available at <http://www.sfk-online.net>. Accessed January 2012.
- Tres A., Bou R., Codony R., Guardiola F. 2008. Influence of different dietary doses of n-3- or n-6-rich vegetable fats and  $\alpha$ -tocopheryl acetate supplementation on raw and cooked rabbit meat composition and oxidative stability. *J. Agr. Food Chem.*, 56: 7243-7253. doi:10.1021/jf800736w
- Ulbricht T.L.V., Southgate D.A.T. 1991. Coronary heart disease: seven dietary factors. *Lancet*, 338: 985-992. doi:10.1016/0140-6736(91)91846-M
- Vani N.D., Modi V.K., Kavitha S., Sachindra N.M., Mahendrakar N.S. 2006. Degradation of inosine 5'-monophosphate (IMP) in aqueous and in layering chicken muscle fibre systems: Effect of pH and temperature. *LWT-Food Sci. Technol.*, 39: 627-632. doi:10.1016/j.lwt.2005.05.003
- Webb E.C., O'Neill H.A. 2008. The animal fat paradox and meat quality. *Meat Sci.*, 80: 28-36. doi:10.1016/j.meatsci.2008.05.029
- Yalçın S., Onbaşlar E.E., Onbaşlar İ. 2006. Effect of sex on carcass and meat characteristics of New Zealand White rabbits aged 11 weeks. *Asian Austral. J. Anim.*, 19: 1212-1216.
- Zhang W., Xiao S., Samaraweera H., Joo Lee E., Ahn D.U. 2010. Improving functional value of meat products. *Meat Sci.*, 86: 15-31. doi:10.1016/j.meatsci.2010.04.018