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INTAKE LIMITATION STRATEGY AND DIETARY PROTEIN CONCENTRATION: EFFECT ON RABBIT GROWTH PERFORMANCE AND HEALTH, FROM A LARGE-SCALE STUDY IN A FRENCH NETWORK OF EXPERIMENTAL UNITS (GEC)

Gidenne T.[1*], Combes S.[1], Briens C.[2], Duperray J.[3], Mevel L.[4], Rebours G.[4], Salaün J.M.[5], Weissman D.[6], Combe Y.[1,7], Travel A.[7]

[1] INRA, UMR1289 TANDEM, Ch. Borderouge, BP 52627, 31326 Castanet-Tolosan, France.
[2] CCPA, ZA du Bois de Teillay, 35150 Janzé, France.
[3] Evialis, Talhouet BP 234, 56006 Vannes, France.
[4] TECHNA, BP 10, rte de St Etienne de Montluc, 44220 Coueron, France.
[5] CYBELIA-Sanders, Centre d'affaires l'Odyssée, ZAC Cicé Blossac, 35170 Bruz, France.
[6] INZO, Rue de l'église, BP 50019, 02407 Chierry, France.
[7] ITAVI, INRA, UMT BIRD, Unité Rech. Avicoles, BP 1, 37380 Nouzilly, France.
[*]Corresponding author: thierry.gidenne@toulouse.inra.fr

ABSTRACT

The performance and the digestive health status of growing rabbits were analysed in five experimental sites, according to a 2x2 design: increasing the dietary crude protein (CP) concentration (C *vs.* HP diet = +20% CP), and free intake or a 25% limitation (100 *vs.* 75) for four weeks after weaning (35d), corresponding to four groups of 541 rabbits (C100, HP100, C75, HP75) bred in collective cages from 35 to 70d. No significant interactions were found between these two factors. From 35 to 70d, a higher dietary CP did not promote the growth or the intake, and even impair slightly the feed conversion (P=0.02). Reversely, a 25% intake limitation improved the feed conversion by 0.4 units (P<0.001) while the growth was reduced by 14% (P<0.001), during the restriction period (35-63d). When restricted rabbits were fed freely (63-70d) a high compensatory growth was recorded for previously restricted rabbits, and was higher for C75 (+21% *vs.* C100) than for HP75 (+14% *vs.* HP100). Over the whole fattening period (35-70d), after a 4 weeks intake reduction (-25%) and one week with a free intake, the growth and final weight were reduced by 10% and 5.6%, respectively (P<0.001), whereas the feed conversion was improved by 0.3 units (P<0.001).
The crude protein level did not affect the digestive health of the growing rabbit. In return, our restriction strategy resulted in a 70% less mortality rate (2.4 *vs.* 7.0%, P<0.001) and a 30% reduction of the morbidity rate (6.2 *vs.* 8.9%, P=0.02) by digestive disorders during the restriction period; accordingly the health risk index was half reduced for restricted rabbits. From weaning to slaughter, the mortality rate decreased from 8.2% for *ad-libitum* fed to 3.3% for restricted rabbits.

Key words: Feed restriction, dietary protein, growth, health, rabbit

INTRODUCTION

Strategies of short-term post-weaning intake limitation are now used by 95% of French rabbit breeders, since it improved the digestive health of the young and also lead to a better feed efficiency (Gidenne *et al.*, 2009). Thus, in 2003, the French network on rabbit experimentation "GEC" that gathered experimental units of 7 private feed companies, an extension centre (ITAVI) and a research centre (INRA), recommend to reduce the dietary level of at least 20%, to reduce mortality from post-weaning digestive disorders, especially during episodes of rabbit epizootic enterocolitis "REE" (Boisot *et al.*, 2003). If these strategies are *a priori* favourable to reduce environmental releases, however they are unfavourable for growth and slaughter yield (Gidenne *et al.*, 2009). To answer to these two questions (releases and carcass yield), the GEC conducted a large scale study, on five experimental sites, to analyze the combined effects of limiting the intake and increasing the dietary protein level (15 *vs.* 18%) on the carcass quality and slaughter yield (Travel *et al.*, 2011), on digestion and nitrogen output (Gidenne *et al.*, 2011), and on growth and digestive health (this paper). Such a large scale study allow to reach a large number of rabbits in several breeding conditions, and thus permit a more precise measure of the feeding level effects on digestive health, as previously done for fibre or starch effects (Gidenne *et al*., 2004, 2005).

MATERIALS AND METHODS

Diets, animals and experimental design

Two diets for growing rabbits (Table 1) were formulated: a control diet "C" with a moderate crude protein concentration (147 g/kg), and an "enriched" protein diet "HP" (180 g/kg, +22%). They were pelleted at one time (Evialis, Talhouet, France) using one batch of raw materials, and without any antibiotic or coccidiostatic. A two factorial design (2x2) was used to differentiate the effect of feeding level "*ad libitum* = 100%" *vs.* "75%" (codes 100 and 75) from that of the dietary crude protein concentration "C *vs.* HP".

Table 1: Ingredients and composition of experimental diets.

Ingredients (%)	C	HP	Analysed composition (g/kg as fed basis)	C	HP
Wheat	3.00	5.20	Humidity	116	115
Barley	7.10	3.00	Crude ash	73	72
Amyplus®	9.50	3.10	Crude protein	147	180
Wheat bran	23.92	24.96	Crude fat	29	26
Soya bean meal 48		6.60	Crude fibre	148	156
Rapeseed meal	3.00	3.00	Starch	115	102
Sunflower meal 32	9.00	19.00	NDF	354	342
Beet pulp	17.50	13.00	ADF	185	188
Lucerne meal 17	11.80	10.00	ADL	56	56
Soya hulls	3.10	2.00	Lysine	78	77
Grape pulp	6.00	4.40	Methionine	31	27
Cane molasse	4.00	4.10	Threonine	65	67
Calcium carbonate	0.23	0.79	*Nutritive value for rabbits fed ad libitum**		
Salt	0.35	0.35	Digestible protein	94	127
L Lysine HCl 78%	0.27		Digestible energy (MJ/kg)	9.52	9.73
DL Methionine 8%	0.14		*Nutritive value for restricted rabbits **		
Threonine 25%	0.59		Digestible protein	108	133
Premix	0.50	0.50	Digestible energy (MJ/kg)	10.38	10.38

*: measured between 42 and 46d old (Gidenne *et al*., 2011).

This concerted study managed by the GEC consisted in replicating within a three months period, the same protocol in 5 experimental sites in France (ITAVI Rambouillet, Euronutrition-SAS Sourches, Evialis St Nolff, INZO Montfaucon, PRIMEX Languidic), to obtain results transposable to commercial breeding. In the 5 sites, rabbits were housed in collective wire net cages, with the same density (0.06 m² per rabbit, 6 to 8 rabbits per cage), in closed units where environment (temperature, ventilation) was controlled. However, the experimental conditions differed in each location (Table 2). A total of 2164 rabbits were allotted at weaning, according to litter origin and live weight, in four groups namely: C100, HP100, C75, HP75. For restricted groups (C75, HP75), the feed was manually given daily between 8:00 and 9:00, during the 4 weeks after-weaning (35 to 63d). The intake level was constant and adjusted (about two times per week) from the intake of the groups fed freely (C100 *vs.* C75, and HP100 *vs.* HP75). Then from 63 to 70d old all groups were fed *ad libitum*. Rabbits were individually identified and weighed at weaning, 49, 63 and 70d old. Feed consumption was measured per period and per cage.

Table 2: Experimental conditions according to the sites.

Site number	Rabbits per group	Cages per group	Rabbits per cage	Genotype	Age at weaning (d)	Age at end of restriction	Age at end of trial (d)
1	140	20	7	Hyplus	36	64	71
2	96	16	6	Hyplus	35	63	68
3	65	13	5	Hyplus	36	63	70
4	132	22	6	Hyplus	35	63	70
5	108	18	6	Hycole	35	63	70

Mortality was measured daily. In the event of mortality, the food remaining in the feeder was weighed to calculate the real feed intake, taking into account the duration of presence and the number of rabbits remaining by cage. Cages having mortality higher than 50% were not included in the statistical analysis of intake and growth performance. Morbidity was measured individually at 49, 63 and 70d old, and consisted in an external observation of all clinical signs of digestive troubles (diarrhoea, caecal impaction, ...) that allowed identifying colibacillosis or epizootic rabbit enteropathy (Licois *et al*., 2005). Animals without visible digestive troubles, but showing severe disturbances of growth (loss of weight during a week, or with abnormally low growth) were also classed as morbid. The morbidity rate was the expression of the number of ill rabbits on the initial number of animals. A rabbit was accounted morbid only one time (within a period) even if diarrhoea may last several days. Therefore, we calculated a health risk index (HRi) corresponding to the sum of morbid and dead animals, knowing that each animal was accounted only once and categorised either dead or morbid (Gidenne *et al*., 2004).

Chemical analyses

The following chemical analyses were carried out on feed (EGRAN, 2001): DM (24 h at 103°C), ash (5 h at 550°C), gross energy (adiabatic calorimeter PARR), fibres (NDF, ADF and ADL) according to the sequential method of Van Soest *et al.* (1991) with an amylolytic pre-treatment. Starch in the feed was hydrolysed enzymatically and the resulting released glucose was measured using the hexokinase glucose-6-phosphate dehydrogenase system (D-Glucose®, Boëhringer Mannheim, Germany).

Statistical analyses

The results of feed intake and growth were first analysed by sites, using a within group variance analyses to detect rabbits having abnormally low growth and to class them as morbid. This was followed by an overall data analysis from the 5 sites (MIXED procedure of SAS), taking into account the effects of the site, the level of restriction (100% or 75%) and the protein content (C vs. HP) and the interactions. Data from morbid animals were maintained in the performances analysis. In addition, the 4 group means were compared by a monofactorial variance analysis and using the test of Scheffe. Data of mortality and morbidity were analysed according to the method of K Pearson (CATMOD procedure of SAS).

RESULTS AND DISCUSSION

No significant interactions were detected among the effect of the site (always significant) and the two main effects (intake and protein levels), thus group means were presented although statistics were given according to the 2x2 design.

As scheduled, a 25% reduction of the intake was achieved from 35 to 63d in C75 and HP75 groups (table 3), and this led to a same weight gain reduction of 14% for C75 or HP75 groups (-6.6 g/d, P <0.001), and to a 7.4% lower live weight at 63d. Thus, during the restriction period, the feed conversion was improved by 0.4 units (-14.3%, P<0.001) that corresponded to a better digestibility of these diets (Gidenne *et al*., 2011), and as generally found in similar trials (Gidenne *et al*., 2012). During the *ad libitum* feeding period (63-70d.), previously restricted rabbits increased their intake by 15% similarly with C or HP diet compared to those fed freely from weaning, as previously reported

(Gidenne *et* al., 2009; Romero *et al.*, 2010). Accordingly, a high compensatory growth was recorded for previously restricted rabbits, that was higher for C75 (+21% *vs.* C100) than for HP75 (+14% *vs.* HP100) group. Finally, over the whole fattening period (35-70d), after a 4 weeks intake reduction (-25%) and one week with a free intake, the growth was reduced by 10%, equally with C or HP diet, whereas the feed conversion was improved by 0.3 units. The final live weight (70d) was 5.6% lower in restricted groups.

Table 3: Intake and growth of rabbits* fed *ad libitum* or restricted, with a control or high protein diet.

Intake:	Ad-libitum		Restricted		*rCV, %*	*Pr> F*		
Groups :	C100	HP100	C75	HP75		*Protein*	*Intake*	*Prot. X Int.*
Feed intake, g/d								
35 to 63 days	146.5 [µ]	148.1 [µ]	109.7	109.8	*5.9 [µ]*	*NC*	*NC*	*NC*
63 to 70 days	177.3 [b]	177.9 [b]	204.6 [a]	204.6 [a]	*7.0*	*0.87*	*<0.001*	*0.80*
35 to 70 days	160.8 [a]	162.4 [a]	134.7 [b]	134.7 [b]	*4.3*	*0.28*	*<0.001*	*0.73*
Live weight, g								
35 days (weaning)	1049	1048	1049	1048	*7.8*	*0.73*	*0.96*	*0.96*
63 days	2451 [a]	2447 [a]	2266 [b]	2270 [b]	*7.9*	*0.78*	*<0.001*	*0.46*
70 days	2705 [a]	2696 [a]	2562 [b]	2546 [b]	*7.8*	*0.18*	*<0.001*	*0.93*
Weight gain, g/d								
35 to 63 days	50.2 [a]	50.0 [a]	43.4 [b]	43.5 [b]	*13.6*	*0.74*	*<0.001*	*0.53*
63 to 70 days	37.7 [c]	37.0 [c]	45.6 [a]	42.3 [b]	*22.7*	*<0.01*	*<0.001*	*0.094*
35 to 70 days	47.9 [a]	47.6 [a]	43.7 [b]	43.1 [b]	*12.6*	*0.13*	*<0.001*	*0.68*
Feed conversion								
35 to 63 days	2.94 [a]	2.96 [a]	2.54 [b]	2.52 [b]	*4.4*	*0.93*	*<0.001*	*0.17*
63 to 70 days	4.84 [ab]	5.00 [a]	4.46 [b]	4.80 [ab]	*20.1*	*0.020*	*0.035*	*0.41*
35 to 70 days	3.36 [a]	3.41 [a]	3.07 [b]	3.08 [b]	*3.6*	*0.024*	*<0.001*	*0.45*

*: including morbid animals; rCV: residual coefficient of variation, according to the bifactorial variance analysis. µ: comparison of C100 *vs.* HP100 only; NC: not calculable (null variance for restricted groups); a, b: means with a common letter did not differ at the level P<0.05.

Table 4: Health status of rabbits* fed *ad libitum* or restricted, with a control or high protein diet.

Intake:	Ad-libitum		Restricted		*Pr> F*		
Groups :	C100	HP100	C75	HP75	*Protein*	*Intake*	*Prot. X Int.*
Mortality rate, %							
35 to 63 days	6.8 [a]	7.2 [a]	2.2 [b]	2.6 [b]	*0.64*	*<0.001*	*0.83*
35 to 70 days	8.1 [a]	8.3 [a]	3.0 [b]	3.5 [b]	*0.62*	*<0.001*	*0.71*
Morbidity rate, %							
35 to 63 days	8.1	9.6	6.1	6.3	*0.52*	*0.02*	*0.62*
35 to 70 days	11.5	11.5	11.6	13.1	*0.61*	*0.52*	*0.61*
Health risk index (HRi), %							
35 to 63 days	15.0 [a]	16.8 [a]	8.3 [b]	8.9 [b]	*0.44*	*<0.001*	*0.80*
35 to 70 days	19.6	19.8	14.6	16.6	*0.46*	*0.01*	*0.53*

* n=541 rabbits per group, mortality and morbidity originated only from digestive disorders; a, b: values with a common letter did not differ at the level P<0.05.

The effect of a 20% increase of the dietary protein level was not significant on intake and growth performances, except during the last week of fattening, when the rabbit fed the high-protein diet showed a 7% lower growth compared to those fed the control diet. Accordingly, the feed conversion was worse for the rabbits fed the HP diet, particularly at this 5th week of fattening but also for the whole fattening period, and this was agreement with Maertens *et al.* (1997). However, we cannot exclude that the lack of a positive growth response for rabbits fed the high-protein diet might be due to an unbalance of the digestible amino-acid proportions.

REE was identified on a low number of cases (< 10 cases) in four sites, while one site declared more than 20 cases of REE and decided to apply antibiotherapy in drinking water with zinc bacitracin, for the four groups and during 7 days (at 43d old). The crude protein level did not affect the digestive health of the growing rabbit. In return, the post-weaning intake limitation resulted in 70% less mortality rate by digestive disorders (2.4 *vs.* 7.0%, P<0.001) and 30% reduction of the morbidity rate (6.2 *vs.* 8.9%) during the restriction period (Table 4); accordingly the HRi was half reduced for restricted rabbits. From weaning to slaughter the mortality rate decreased from 8.2% for *ad-libitum* fed to 3.3% for restricted rabbits.

CONCLUSIONS

In conclusions, increasing the dietary protein level by 20% did not improve the performances and had no negative impact of the digestive health. In return, limiting the intake after weaning led to a better digestive health status, and to a better feed efficiency. In the conditions of the French market, such a restriction strategy led to significant economic benefit estimated at 24ct € per weaned rabbit.

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