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Effects of feed particle size on caecal activity and growth performances in fattening rabbits

Progress in Nutrition
Vol. 9, N. 3, 189-197, 2007

Titolo
Prestazioni produttive e caratteristiche del contenuto ciecale di conigli alimentati con diete a diverso profilo granulometrico

Key words
Rabbit, feed particle size, growth performances, caecal content, volatile fatty acids

Parole chiave
coniglio, granulometria mangime, prestazioni produttive, contenuto ciecale, acidi grassi volatili

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Summary
Aim of this work was to investigate the effects of particle size of feeds for fattening rabbits on growth performances, slaughtering data and, particularly, on their caecal fibrolytic activity and caecal content characteristics. 72 hybrid commercial rabbits 40 days old, divided in two experimental groups, were fed to slaughter (88 days) two pelletted diets (F – fine; C – coarse) of similar composition differing only in the grinding level of dehydrated lucerne meal and wheat bran. 42.3% of diet F was composed by particles with ∅<0.315 mm, while these particles were only 31.7% of diet C (P<0.001). The smallest particle class (∅<0.315 mm) showed 58.6% of NDF and 33.0% of ADF in diet F, while NDF was 53.0% and ADF 26.2% in C diet (P<0.001). Mortality rate, growth performances, feed consumption, and feed/gain ratio never differed between the two diets. No effect could be registered concerning carcass weight, dressing out percentage, full gastrointestinal weight and caecum weight. As for the caecal chemical composition, volatile fatty acids concentration and their proportion, no significant differences were registered between F and C diets, and pH averaged very similar values in both groups (6.24 vs 6.30 for F and C respectively). Also enzymatic activity into the caecum, as fibrolytic and amylolitic response, was the same. So, a minute milling of different raw materials, does not appear a reliable way to significantly modify gut conditions and subsequent rabbit performances.

Riassunto
Obiettivo della ricerca è stato quello di valutare l'effetto del diverso profilo granulometrico del mangime sui parametri produttivi e le caratteristiche del contenuto ciecale di conigli in accrescimento. Per la prova sono stati utilizzati 72 conigli, ibridi commerciali, svezzati all'età di 40 giorni e ripartiti in due gruppi omogenei. Gli animali hanno ricevuto, per l'intero periodo sperimentale (48d), due mangimi pellettati (F = fine; C = grosso), di analoga composizione e che differivano unicamente per il grado di macinazione di alcune materie prime (medica disidratata e crusca di frumento).
Il 42,3% del mangime F era costituito da particelle con ∅<0,315 mm, mentre queste erano solamente il 31,7% nel mangime C (P<0,001). Le particelle appartenenti alla classe più fine del mangime F (∅<0,315 mm)

presentavano il 58,6% di NDF e il 33,0% di ADF mentre, nel mangime C, avevano il 53,0% di NDF e il 26,2% di ADF (P<0,001). L'incidenza della mortalità e le prestazioni produttive in vivo non sono state influenzate dal diverso profilo granulometrico dei mangimi. Non sono state registrate differenze tra i gruppi relativamente ai parametri produttivi rilevati in sede di macellazione, al peso del digerente ed a quello del cieco. La composizione chimica del contenuto ciecale, il valore del pH (6,24 vs 6,30 per F e C, rispettivamente) e le proporzioni molari degli A.G.V. non si sono espresse in maniera significativamente diversa tra le tesi sperimentali. Anche l'attività enzimatica del contenuto ciecale, in particolare quelle fibrolitica e amilolitica, sono risultate analoghe tra i trattamenti. Benché la tecnica di macinazione impiegata abbia modificato significativamente il profilo granulometrico dei mangimi e la composizione chimica delle diverse frazioni, questo risultato non ha influito sulle condizioni del digerente e di conseguenza sulle prestazioni produttive degli animali.

Introduction

The digestive activity of the rabbit presents several peculiarities. In particular, it is proved that antiperistaltic movements, induced by small size feed particles (∅<0.315 mm), take place in the proximal colon during hard faeces production (1). These particles, carried back to the caecum, are subdued to a longer digesta mean retention time (MRT) (2) and cause a higher repletion level. These fine particles could be relevant in connection to soft faeces formation activity. In fact, Sakaguchi et al., (3) demonstrated that the deprivation of caecotrophy could cause a shortening of the feed particles' MRT and a reduction in NDF and ADF digestibility only with diets rich in small size feed particles. Furthermore small particles may have a modulating effect on the caecal microbial flora related to their chemical composition. As stated in a previous work (4), one effect of different feed grinding fineness is a change in cell wall polysaccharides digestibility in relation to the different particle sizes. Aim of the present research was to investigate the relation between feed particle size, fibrolytic activity and caecal content characteristics. The effects of feed grinding fineness on growth performances, slaughtering data and mortality were also registered.

Materials and methods

For this trial 72 hybrid commercial rabbits (half males and half females) were used. The animals were homogeneously divided in two experimental groups of 36 each one. From weaning (40 days) to slaughter (88 days) the rabbits were housed in two-place cages (cm 24 x 41 x 28 h) and fed, *at libitum*, two pelletted diets (∅ = 3.5 mm, length= 10 mm) of similar composition (Tab. 1) (5), differing only in the grinding level of some constituents. In the feed provided to rabbits in group F (Fine), dehydrated lucerne and wheat bran had undergone double grinding (1st milling with a ∅ = 5 mm grinder, 2nd milling with ∅ = 1 mm), while rabbits in group C (Coarse) received a feed whose constituents had been ground only once (∅ = 5 mm grinder).
The feed particle size and distribution in the different size classes were determined on feed, after mixing the constituents and before pellet-

Table 1 - Composition of the experimental diets

Diets	F	C
Chemical composition (% D.M.):		
Dry matter (%)	91.8	92.53
Crude protein (N x 6.25)	17.46	18.09
Crude lipids	3.45	3.31
Crude fibre	15.32	14.29
Ash	8.79	8.56
Neutral detergent fibre	34.15	34.71
Acid detergent fibre	19.11	18.15
Acid detergent lignine	3.19	3.17
Digestible energy (Mj/Kg)*	10.5	10.8

Ingredients (%): dehydrated lucerne meal (40.0), wheat bran shorts (20.0), barley (11.0), wheat bran (10.0), soybean meal (8.0), wheat middlings (8.0), sugarcane molasses (1.4), dicalcium phosphate (0.5), sodium chloride (0.3), methionine (0.5), minerals and vitamins (0.3), *robenidine (66 mg/Kg)*
* Calculated (5)

ting. This measurement was carried out following a dry method using normalised siever with square meshes (side of 1, 0.5, 0.315 and 0.125 mm, in sequence) in a RETSCH AS 200 BASIC analytical siever (6 min – 100 amplitude). This procedure was repeated with five 50 g replicates for each diet, in order to perform statistic analysis of the particle size distribution. The same particle size classes of all the 5 replicates were then mixed together and analysed three times in order to determine dry matter [930.15], crude protein [984.13], crude lipids [954.02], crude fibre [978.10], ash [942.05], according to standard methods (6). aNDF, ADL and Lignin (sa) (ADL) were determined according to the method proposed by Goering and Van Soest (7) and using α-amylase SIGMA (cod. 3306).

During the trial, the individual live weight and the feed intake per replicate (2 rabbits) were recorded every two weeks. Dead animals underwent post mortem investigation in order to evidence any pathological features. At the end of the trial all the rabbits were individually weighed and slaughtered without any fasting period. For each one, the weight of the full gastrointestinal tract and of the hot carcass (inclusive of the head, kidneys, liver, hearth, lungs, scapular and perirenal fat) were recorded. Furthermore, from 20 rabbits, ten from each group, randomly chosen, the caecum with its content was separated and weighed. The total caecal content, after a thorough mixing, was immediately used for measuring the pH value. Afterwards, the caecal content was divided in three portions. One of these was used to determine the chemical composition (AOAC, 1990). The second fraction (2,5 g weight) was centrifuged (5 minutes at 5000 r.p.m.) and filtered. The aqueous extract was used to determine the volatile fatty acids (VFA) content following a gas-chromatographic method, using crotonic acid as internal standard (8). For this a NUKOL SUPELCO capillary column (15m length, 0.25 m film thickness, 0.25 mm inner diameter) was employed, working from 80°C to 195°C (8°C/min) for a total of 25 minutes, with injector at 240°C and detector at 250°C and using a ionised flame detector. A mix of VFA (SUPELCO Volatile Acid Standard MIX 46975-U) at known concentration was used to calculate the correction factors.

The last fraction (3 g weight) was diluted in 20 mM phosphate buffer pH 7.0 containing 0.5 mM of a protease inhibitors solution. Samples were stored at -20°C until the enzymatic activity determination. Protein concentrations were determined using the Bradford method (9).

Determination of cellulase activity

The assay was carried out using 1 g

of rabbit caecal content, which was incubated at 25°C for 48 h with 200 mg of carboxymethylcellulose in 5 ml of 40 mM acetate buffer pH 4.8, with constant gentle mixing. Then, samples were centrifuged at 15.000 x *g* for 15 min using a ALC micro Centrifugette 4214 (International pbi, Milano, Italy). To evaluate the hydrolytic activity surnatants were used . One enzymatic unit was defined as the amount of enzyme able to form 1 mmole of glucose in one hour at 37°C.

Xylanase assay

This assay was performed using birchwood xylan as substrate dissolved in 0.2 M acetate buffer pH 5.5. Reducing sugars were determined as described in the following section. One enzymatic unit was defined as the amount of enzyme able to form 1 mmole of xylose per min at 30°C (10).

Determination of reducing sugars

The soluble products (reducing sugars) obtained after the two abovementioned enzymatic hydrolysis were determined using the ferricyanide method (11). The latter compound is reduced by sugars to form ferro cyanide that reacting with ferric alum forms a stable blue color determined spectrophotometrically at 710 nm.

α-amylase assay

For the determination of the amylase activity [EC 3.2.1.1] a Sigma Diagnostic Amylase Reagent was used, that allows a quantitative kinetic determination of a-amylase activity following the increase of the absorbance at 405 nm. To perform this assay 1 g of caecal content was dissolved in 5 ml of 0.1 M sodium phosphate buffer pH 7.0, the homogenized for 10 min using a Potter-Elvehjem and finally centrifuged at 15.000 xg for 15 min. The surnatant was used for the enzyme assay.
All enzymatic assays were performed using a Beckman DU 640 Spectrophotometer.
All the data, except those concerning the pathological investigation and feed particle size distribution and composition, were analysed according to the GLM procedure of the SAS statistical package (12) and applying the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk};$$

where:
Y = dependent variable
μ = mean
α = treatments (i: F = fine; C = coarse)
β = sex (j: males and females)
ε = error

The model included the effects of treatment and sex, without interaction because not significant. The differences between males and females were always very slight and are consequently not reported in the tables. When the F test analysis of variance was significant ($P<0.05$), differences among means were compared using the SNK test. Mortality rate between the experimental groups was compared using the χ^2 test.

Results

Characteristics of the diets

The different milling of dehydrated lucerne and wheat bran in the two different diets influenced significantly particle size distribution (Tab. 2). All the particle size classes were significantly different between the two experimental diets. Specifically, 42.3% of diet F was composed by particles with ∅<0.315 mm, while these particles were only 31.7% of diet C ($P<0.001$). As expected, also chemical composition of the different size classes was significantly influenced by the different milling, particularly as for fibre content (Tab. 3). The smallest particle classes (∅<0.315 mm) showed 29.3% of NDF and 16.7% of ADF in diet F, while NDF was 26.5% and ADF 12.9% in C diet ($P<0.001$). The finest particles had the same ADL content (3.8 *vs* 3.8 for F and C diet respectively).

Table 2 - Particles distribution among the size classes for the experimental diets (means ± s.d.)

Particle size (mm)		Diets		P values
		F	C	
>1	%	10.0 ± 0.5	23.7 ± 1.3	< 0.001
0.5÷1	%	27.3 ± 0.6	28.2 ± 0.4	0.026
0.315÷0.5	%	20.4 ± 0.4	16.4 ± 0.3	< 0.001
0.125÷0.315	%	30.4 ± 0.4	22.8 ± 1.1	< 0.001
<0.125	%	11.9 ± 0.4	9.0 ± 0.6	< 0.001

Table 3 - Fibre composition of the different particle size classes of the experimental diets (means ± s.d.)

Particle size (mm)		Diets		P values
		F	C	
>1	NDF (%)	21.1 ± 0.6	29.5 ± 0.3	<0.001
0.5÷1		38.4 ± 0.3	40.4 ± 0.1	<0.001
0.315÷0.5		39.2 ± 0.4	36.9 ± 0.3	0.001
0.125÷0.315		34.7 ± 0.2	31.1 ± 0.3	<0.001
<0.125		23.8 ± 0.5	21.9 ± 0.4	0.006
>1	ADF (%)	9.3 ± 0.1	16.6 ± 0.1	<0.001
0.5÷1		19.8 ± 0.2	22.2 ± 0.2	<0.001
0.315÷0.5		20.0 ± 0.1	17.7 ± 0.8	0.007
0.125÷0.315		19.6 ± 0.1	15.2 ± 0.3	<0.001
<0.125		13.7 ± 0.4	10.6 ± 0.1	<0.001
>1	ADL (%)	2.5 ± 0.03	4.2 ± 0.5	0.003
0.5÷1		4.2 ± 0.2	5.5 ± 0.1	<0.001
0.315÷0.5		4.4 ± 0.1	4.5 ± 0.3	0.445
0.125÷0.315		4.3 ± 0.3	4.5 ± 0.1	0.349
<0.125		3.2 ± 0.3	3.1 ± 0.04	0.361

Mortality and growth performances

Mortality rate averaged 15.3%, and death causes were mainly enteric problems as stated by necropsy. Particularly, there were 3 (8.3%) and 8 (22.2%) dead rabbits for group F and C respectively, difference that did not reach a significant level (P=0.10), according to the χ^2 test. Similarly, growth performances, feed consumption, and feed/gain ratio never differed between the two diets (Tab. 4).

Slaughtering data and gastrointestinal characteristics

Carcass weight and dressing out percentage (Tab. 4) were not different between the two treatments. In the same way, the full gastrointestinal weight and the caecum weight were the same for the two diets (Tab. 5). As for the caecum chemical composition, no significant differences were registered between F and C diets, and pH averaged very similar values in both groups (Tab. 6). No effects of the diets could be noted on the caecal volatile fatty acids concentration and their proportion, and enzymatic activity into the caecum, as fibrolytic and amylolitic response, was the same (Tabb. 6, 7).

Discussion

The dry-sieving method we used for granulometric profiles' evaluation on feed before pelletting was characterized by a high "repeatibility" and "reliability". Lebas and Lamboley (13) suggested to use a liquid phase sieving that however give particle size profiles, obtained before or after pelleting, very different each others and also compared to that determined by dry method on flour. Lebas et al. (14) suggest

Table 4 - Growth performances and slaughtering data of the rabbits fed diets differently ground (mean values)

Parameters		Groups		S.D. error (1)	P values
		F	C		
Live weight at weaning	g	1192 (36)	1190 (36)	94.9	0.90
Live weight at slaughter	g	2993 (33)	2896 (28)	312	0.21
Daily weight gain	g	37.5	35.5	5.7	0.14
Daily feed intake	g	153.9	146.3	13.9	0.16
Feed to gain ratio	-	4.19	4.32	0.53	0.30
Hot carcass weight	g	1849	1779	207	0.16
Dressing percentage	%	61.7	61.4	1.6	0.24

In parenthesis: number of animals; (1) Standard Deviation of error

Table 5 - Caecal weight and chemical composition of its contents of the rabbits fed diets differently ground (mean values)

Parameters		Groups		S.D. error	P values
		F	C		
Number of replicates	-	10	10	-	-
Slaughtering weight	g	2941	2955	292	0.99
Full gastrointestinal tract weight	g	471	460	44	0.47
Caecal weight	g	179	169	20	0.20
Caecal content composition:					
Dry matter	%	23.3	22.6	1.8	0.39
Crude protein	%	26.6	27.8	2.5	0.23
NDF	%	51.6	52.2	2.2	0.72
ADF	%	27.3	28.1	2.9	0.55
ADL	%	11.8	12.6	2.9	0.57

that pelleting has an evident effect on the grinding fineness, but also ultrasounds and water used in liquid phase sieving seem to have remarkable consequences, probably modifying the real particle size profile. Furthermore, the liquid phase sieving imply the solubilisation of some particles giving a profile that should be very different than real and does not allow to determine the chemical composition of some particle classes, particularly the finest and more soluble ones. The use of dry-sieving on feed before pelletting was then necessary considering the objectives of ours research. In fact, this method makes the recovering of the finest fraction and its chemical composition's determination possible.

The results recorded on particle size distribution and on chemical composition of the different size classes indicate that the finest grinding acted with different effectiveness on structural polysaccharides of raw materials, as already observed in a previous research (4).

Mortality rate, even if higher than usually observed in the farm where the trial was carried out (<10%), did not seem to be influenced by grinding fineness. Morisse (15) described a reduction in mortality rate in rabbits fed with finely ground feeds, while other findings didn't report any effects in the same conditions (14). In any case, in the present study neither necropsy nor observations performed on rabbits normally slaughtered could demonstrate any problem ascribable to the different treatments.

With reference to growing performances and feed efficiency, as already observed in a previous study (4), the different particle size distribution and the higher cellulose percentage of the finest class of the F diet did not achieve have any influence. Some authors could relate of better growth results in connection with more finely ground feeds

Table 6 - pH and Volatile Fatty Acids composition of caecal content of the rabbits fed diets differently ground (mean values)

Parameters		Groups		S.D. error	P values
		F	C		
Number of replicates		10	10	-	-
pH	-	6.24	6.30	0.29	0.53
Volatile Fatty Acids concentration	mg/g	3.17	3.72	0.76	0.10
Volatile Fatty Acids (mol/100 mol):					
Acetic ac.		76.81	77.94	1.99	0.42
Propionic ac.		6.60	6.11	0.93	0.28
Isobutyric ac.		0.51	0.56	0.23	0.86
Butyric ac.		14.74	14.23	2.29	0.93
Isovaleric ac.		0.46	0.45	0.23	0.79
Valeric ac.		0.87	0.71	0.21	0.10

Table 7 - Enzymatic activity of caecal content of the rabbits fed diets differently ground (mean values)

Parameters	Groups		S.D. error	P values
	F	C		
Number of replicates	10	10	-	-
Cellulase (μmol/g/h)	1.31	1.18	0.26	0.39
Xylanase (μmol/g/h)	1.16	1.16	0.10	0.99
α-Amylase (μmol/g/h)	54.78	80.31	33.68	0.06

(16, 17), while other observed any effect (18, 19). Different findings are certainly to be related to the high heterogeneity in the grinding fineness adopted in the trials conducted in the past, while even particle size distribution is not always clearly defined in the studies.

With regard to slaughtering data, the dressing out percentage was not influenced by the treatments; on the contrary, other authors could find a higher incidence of the full gastrointestinal tract in rabbits fed finely ground diets (15, 17).

The hypothesis that the finest grinding of raw fibrous materials should determine a higher repletion of the caecum, as a consequence of antiperistaltic movements in the proximal colon due to small particles (∅<0.315 mm) (1), and a longer digesta mean retention time (MRT) (2) was not confirmed by the findings related to weight, chemical composition and enzymatic activity of the caecum.

Considering mean daily feed intake, undoubtedly rabbits of the F treatment were fed a greater amount of fine particles: 66.2 g/d *versus* 47.7 g/d received by rabbits of the C diet [(daily feed intake) x (% particles ∅<0.315 mm)]. It has been demonstrated that minute milling of raw material allows digestion of the fibrous components, particularly hemicellulose and pectins, already in the first tract of the bowel (16). This could partly explain the lack of results, even though in our research the finest fraction was principally composed by cellulose (ADF-ADL) which should reach the proximal colon being slightly degradable and being the digesta MRT to the ileum quite short in the rabbit (4-9 hours) (20).

Concerning chemical composition and enzymatic activity, the kind of fibre that reaches the caecum is surely able to influence the characteristics of its content. For example, great changes can be registered in fibrolytic activity as well as in volatile fatty acids content in young rabbits following weaning (21, 22); furthermore, fermentation patterns of the caecal contents in growing rabbits are clearly influenced by fasting (23). However, aside from

these extreme changes in feeding, some authors could find only slight changes in VFA caecal content (particularly regarding acetic acid) following a great increase in hemicellulose and pectins in the feed of growing rabbits (6 to 10 weeks of age) (24). Therefore, in our trial the lack of pH and VFA changes could be explained as a consequence of weak changes in fibrous content of the caecum, despite the differences in the feeds. Furthermore, cellulose hydrolysis is a slow process and the rate of passage of this component in the caeco-colic compartment is usually too fast (6 to 10 hours for whole digesta) to allow a complete fermentation (22, 24). The finest grinding adopted for F diet in our trial may not have significantly increased the mean retention time in the caeco-colic compartment, time necessary to allow a greater cellulose fermentation rate. However, Bellier and Gidenne (26), in 6 weeks rabbits fed diets with a greater NDF content, could observe a doubled mean retention time, but without significant variations in pH o VFA caecal content.

At last, it is possible that the differences between the diets, sufficiently clear when mixing the raw constituents, might not have remained the same in the proximal colon as an effect of the digestive processes in the proximal digestive tracts.

Conclusions

The different milling of fibrous raw materials in the two different diets influenced significantly particle size distribution as well as chemical composition of the various size classes. These differences, however, never influenced mortality rate, growth and slaughtering performances, in fattening rabbits. Neither weight, nor enzymatic activity, nor subsequent chemical analysis results for the caecal content were influenced by the treatments. In conclusion, a minute milling of different raw materials, which could imply greater feed production costs, does not appear a reliable way to significantly modify gut conditions and subsequent rabbit performances.

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