Increasing Dietary Energy with Starch and Soluble Fibre and Reducing ADF at Different Protein Levels for Growing Rabbits

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Summary

The effect of increasing dietary energy by increasing both starch (14% to 20%) and soluble fibre (9.6% to 11.6%) and decreasing ADF content (21% to 13%) at two crude protein levels (14% and 15%) was assessed on health, growth performance, caecal fermentative activity, gut mucosa traits, and carcass and meat quality of growing rabbits reared from 34 to 76 days of age. At weaning, 306 rabbits were divided into six groups and fed *ad libitum* six diets formulated according to a bi-factorial arrangement with 3 (starch+soluble fibre)/ADF ratio (L=1.2; M=1.8; H=2.8) x 2 protein levels. The contemporary increase of dietary starch and soluble fibre and the reduction of ADF linearly increased the digestibility of dry matter, energy and all nutrients, decreased feed intake and improved feed conversion (3.65 to 2.76 in rabbits fed diets L and H; P<0.001); it increased morbidity (P=0.09) but did not affect mortality due to epizootic rabbit enteropathy; at caecum, VFA production raised (52.1 to 61.9 mmol/L) and pH (5.90 to 5.67) and N ammonia lowered (4.85 to 1.93 mmol/L); the villi/crypts ratio (4.42 *vs.* 3.81 and 3.95 in rabbits fed diets L, M and H) decreased in a non linear mode. The decrease of the protein level did not affect growth performance, but nearly halved mortality (28.9% *vs.* 16.3%; P=0.01) and increased caecal pH (5.70 to 5.87; P=0.05). Weak effects of the dietary treatments were measured on carcass traits and meat quality.

Key words

starch, soluble fibre, insoluble fibre, protein, growing rabbits

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Received: April 23, 2013 | Accepted: June 26, 2013

ACKNOWLEDGEMENTS
This study was funded by the University of Padova, Italy (2011 – CPDA119830).

Aim

The present study aimed at evaluating whether the increase of dietary energy obtained by a contemporary increment of starch (14% to 20%) and soluble fibre (9.6% to 11.6%) concentration at the expense of ADF supply (21% to 13%) and the variation of protein level (14% to 15%) can affect health status, growth performance, digestive efficiency, caecal fermentation activity and gut mucosa traits, and carcass and meat quality in growing rabbits.

Material and methods

A total of 306 weaned Hyplus crossbred rabbits (Hypharm, Groupe Grimaud, Roussay, France) of both genders were put in individual cages (285 x 410 x 285 mm) at 34 d of age, divided into six groups (51 rabbits each), homogeneous in average live weight and variability, and given *ad libitum* access to six experimental pelleted diets until slaughter (76 d of age). The diets (L14, M14, H14, L15, M15 and H15) were formulated as a combination of three (starch+soluble fibre)/ADF ratios (low, L=1.2; moderate, M=1.8; high, H=2.8) combined with two crude protein levels (14% and 15%) (Table 1). No antibiotics or drugs were included in the feed. Individual live weight and feed intake were recorded three times a week. Health status was controlled daily.

Epizootic rabbit enteropathy (ERE) occurred soon after one week of trial and all rabbits were treated with antibiotics in water (tiamulin and colistin) from 48 to 57 days of age. At 42 d of age, 36 rabbits (6 per diet) out of the 306 initial rabbits were slaughtered to sample caecal content and gut mucosa (jejunum and ileum). The digestibility coefficients and digestible energy (DE) concentration of the experimental diets were measured *in vivo* on 72 rabbits among those on trial (12 animals per diet) from 49 to 52 days of age according to Perez *et al.* (1995). Diets, faeces, caecal contents and gut mucosa were analyzed according to the methods detailed by Trocino *et al.* (2013a). The soluble fibre content was obtained by subtracting NDF after correction for ash and protein from TDF (Trocino et al., 2013b). At 76 d of age, 120 rabbits were selected (20 animals per diet) representative in terms of average weight and variability and slaughtered. After 24 h of cooling at 4°C, the carcasses were dissected according to the international scientific protocols (Blasco et al., 1993). The data of performance, digestibility coefficients, intestinal mucosa traits and caecal fermentation activity were submitted to ANOVA with (starch+soluble fibre)/ADF ratio, protein level and their interaction as main factors and using the GLM procedure of SAS (SAS Institute, Cary, NC, USA). Orthogonal contrasts were used to test the linear and quadratic response to the increase of the (starch+soluble fibre)/ADF ratio. Mortality, morbidity, and sanitary risk (i.e., mortality+morbidity) were analysed by the CATMOD procedure of SAS.

Results and discussion

Despite the occurrence of ERE which severely affected rabbits during the first weeks on trial (average mortality: 22.6%), animals recovered well thanks to the antibiotic treatment and showed satisfying performance for the genetic type used: rabbits weighed on average 2831 g at 76 days of age, with a daily weight gain of 44.1 g/d, a feed intake of 141 g/d and a feed conversion of 3.21 in the whole period of growth. As the interactions were not significant, the results are presented and discussed according to the main factors.

Effect of (starch+soluble fibre)/ADF ratio – When the ratio increased from 1.2 to 1.8 to 2.8, the digestibility of energy and all nutrients linearly raised (Table 2), especially fibre fractions with the exception of soluble fibre (85-90%); therefore the DE concentration increased from 9.7 to 11.0 to 12.2 MJ/kg (Table 1); growth rate did not change (Table 3) but feed intake linearly decreased (P<0.001) both in the first and second period of the

Table 1. Ingredients (%), chemical composition and nutritive value of the experimental diets

Diet	L14	M14	H14	L15	M15	H15
Ingredients						
Dehydrated alfalfa meal 16% CP	37.00	18.50	0.00	36.00	18.00	0.00
Wheat bran	21.00	18.50	16.00	30.00	25.00	20.00
Barley	16.00	24.50	33.00	10.50	20.25	30.00
Dried beet pulp	17.48	25.39	33.30	13.50	21.75	30.00
Soybean meal 50% CP	4.00	7.75	11.50	6.00	10.00	14.00
Sunflower oil	1.00	1.50	2.00	1.00	1.50	2.00
Other ingredients[a]	3.52	3.86	4.20	3.00	3.50	4.00
Chemical composition						
Dry matter, %	89.3	89.1	88.9	89.6	88.9	88.4
Crude protein, %	14.3	14.3	14.2	14.8	15.2	15.1
Ether extract, %	3.39	3.65	4.01	3.81	3.74	4.17
TDF, %	42.7	40.2	37.3	43.7	40.3	35.9
NDF, %	34.7	30.5	26.4	36.3	31.3	26.2
ADF, %	18.4	14.9	11.4	18.9	15.0	11.2
ADL, %	2.7	1.7	0.8	2.9	1.8	0.8
Soluble fibre, %	10.0	11.1	12.0	9.1	10.0	11.2
Starch, %	14.0	17.8	21.2	12.4	16.2	19.6
Digestible energy (DE), MJ/kg	9.8	11.2	12.2	9.6	10.8	12.3
Digestible protein/DE, g/MJ	10.5	9.1	8.6	11.4	10.5	9.4

[a]Molasses, limestone, dicalcium phosphate, salt, L-lysine, DL-methionine, DL-threonine, vitamin-mineral premix.

Table 2. Digestibility coefficients (%)

	(Starch+soluble fibre)/ADF ratio					Protein level			RSD[a]
	1.2	1.8	2.8	P_L[b]	P_Q[c]	14%	15%	Prob.	
Rabbits, n	23	21	22			33	33		
Dry matter	58.2	66.4	75.6	<0.001	0.37	67.1	66.4	0.13	1.86
Crude protein	72.5	73.0	75.4	<0.001	0.08	72.5	74.8	<0.001	2.05
Ether extract	79.2	81.2	85.4	<0.001	<0.01	81.4	82.4	<0.01	2.32
TDF	37.4	49.6	61.6	<0.001	0.92	50.2	50.0	0.14	3.21
aNDF	27.0	37.6	51.0	<0.001	0.14	39.4	37.7	0.07	3.68
ADF	17.8	27.1	40.4	<0.001	0.09	28.3	28.6	0.83	4.41
Soluble fibre	85.5	89.4	88.7	0.12	0.20	85.0	90.8	<0.001	6.61
Starch	97.5	98.0	98.6	<0.001	0.47	98.1	98.0	0.03	0.23
Gross energy	59.0	66.6	75.2	<0.001	0.33	67.5	66.4	0.02	1.99

[a]Residual standard deviation. [b]Linear component of variance. [c]Quadratic component of variance.

Table 3. Performance and health status from 34 d until 76 d of age

	(Starch+soluble fibre)/ADF ratio					Protein level			RSD[a]
	1.2	1.8	2.8	P_L[b]	P_Q[c]	14%	15%	Prob.	
Rabbits, n	71	65	66			108	94		
Live weight, g									
At 34 d	974	987	972	0.92	0.34	986	970	0.27	99
At 55 d	2041	2034	1985	0.13	0.53	2036	2004	0.28	212
At 76 d	2826	2862	2804	0.63	0.23	2829	2832	0.93	255
First period (34-55 d)									
Daily weight gain, g/d	50.5	50.2	47.9	0.13	0.53	50.3	48.7	0.28	10.1
Feed intake, g/d	145	128	107	<0.001	0.54	127	126	0.76	19
Feed conversion	2.89	2.69	2.38	<0.001	0.60	2.56	2.75	0.07	0.72
Second period (55-76 d)									
Daily weight gain, g/d	37.4	39.4	39.0	0.19	0.26	37.8	39.5	0.10	7.2
Feed intake, g/d	175	159	132	<0.001	0.04	152	158	0.05	20
Feed conversion	4.82	4.12	3.44	<0.001	0.90	4.16	4.09	0.51	0.79
Whole trial (34-76 d)									
Daily weight gain, g/d	44.0	44.8	43.4	0.63	0.23	44.0	44.1	0.93	6.1
Feed intake, g/d	160	144	120	<0.001	0.12	140	142	0.33	17
Feed conversion	3.65	3.22	2.76	<0.001	0.65	3.19	3.23	0.28	0.25
Health status[d]									
Mortality, %	21.1	23.3	23.3	0.79		16.3	28.9	0.01	
Morbidity, %	17.8	20.0	31.1	0.09		25.9	20.0	0.32	
Sanitary risk[e], %	38.9	43.3	54.4	0.10		42.2	48.9	0.26	

[a]Residual standard deviation. [b]Linear component of variance. [c]Quadratic component of variance. [d]Probability of χ^2-test. [e]Mortality + morbidity.

trial and, as a consequence, feed conversion improved (P<0.001). The improvement of feed conversion in diets with higher DE obtained increasing starch and soluble fibre in replacement of insoluble fibre was expected (Blas and Gidenne, 2010; Trocino *et al.*, 2013b), whereas the consequences on health status were less obvious. A higher morbidity was observed in rabbits fed the highest (starch+soluble fibre)/ADF ratio (from 17.8 and 20.0% to 31.1%; P=0.09), but mortality was not modified (Table 3). As reviewed by Blas and Gidenne (2010), health of rabbits may impair when starch is increased at the expense of insoluble fibre, while positive effects may be recorded when soluble fibre replaces insoluble fibre, especially in presence of ERE (Xiccato *et al.*, 2006; Gómez-Conde *et al.*, 2007; Martínez-Vallespín *et al.*, 2011). In the present trial, the increase of soluble fibre likely counterbalanced the negative effects on health of increasing starch and decreasing ADF, therefore permitting to use high energy diets without consequences.

With the increase of the (starch+soluble fibre)/ADF ratio, VFA production at caecum linearly increased and pH value and N-ammonia decreased; the proportion of acetic acid tended to raise and that of propionic acid to decrease (Table 4). The major availability of fermentable carbohydrates, both as starch and soluble fibre, promoted caecal VFA production and reduced N-ammonia levels (Jehl and Gidenne, 1996; Blas and Gidenne, 2010; Trocino *et al.*, 2013b), and likely modified caecal microflora, in rabbits fed higher dietary (starch+soluble fibre)/ADF ratio. However, as demonstrated by the higher digestibility, also insoluble fibre may have been more susceptible to microflora fermentation in the caecum, because of the lower lignification and complexity of the cell walls or different hemicellulosic constituents (Carabaño *et al.*, 2001; García *et al.*, 2002). Previous studies correlated improvements in health status with a better barrier function of the intestinal mucosa in rabbits fed higher levels of the most soluble fibre fractions (from 7 to 12%) at the

Table 4. Caecal content and intestinal mucosa traits

	(Starch+soluble fibre)/ADF ratio					Protein level			RSD[a]
	1.2	1.8	2.8	P_L[b]	P_Q[c]	14%	15%	Prob.	
Caecal content									
pH	5.90	5.78	5.67	0.03	0.93	5.87	5.70	0.05	0.24
$N\text{-}NH_3$, mmol/L	4.85	2.59	1.93	<0.01	0.32	2.67	3.58	0.24	2.23
Total VFA, mmol/L	52.1	61.4	61.9	0.03	0.24	56.3	60.6	0.23	10.5
C_2, % mol.	81.8	82.3	84.6	0.07	0.45	82.9	82.8	0.99	3.63
C_3, % mol.	5.19	4.52	4.36	0.09	0.54	4.95	4.44	0.20	1.15
C_4, % mol.	12.5	12.7	10.5	0.18	0.32	11.6	12.2	0.65	3.51
C_5, % mol.	0.57	0.55	0.56	0.87	0.85	0.57	0.55	0.81	0.21
C_3/C_4 ratio	0.45	0.37	0.49	0.66	0.20	0.44	0.43	0.85	0.21
Jejunum mucosa									
Villi height[d], µm	839	733	751	<0.01	0.01	780	768	0.58	64
Crypts depth, µm	192	194	192	0.99	0.69	188	197	0.14	19
Villi/crypts	4.42	3.81	3.95	0.02	0.03	4.19	3.92	0.10	0.47
Ileum mucosa									
Villi height, µm	601	617	660	0.26	0.76	639	612	0.51	125
Crypts depth, µm	148	159	155	0.33	0.23	152	155	0.58	17
Villi/crypts	4.09	3.97	4.27	0.63	0.52	4.24	3.97	0.38	0.91

[a]Residual standard deviation. [b]Linear component of variance. [c]Quadratic component of variance. [d]Villi height: 867, 758, 715, 811, 706 and 786 µm in rabbits fed diets L14, M14, H14, L15, M15, H15; probability of R x P interaction = 0.03.

Table 5. Results at commercial slaughter and carcass quality

	(Starch+soluble fibre)/ADF ratio					Protein level			RSD[a]
	1.2	1.8	2.8	P_L[b]	P_Q[c]	14%	15%	Prob.	
Slaughter weight (SW)[d], g	2756	2788	2748	0.87	0.39	2765	2763	0.96	213
Gut incidence[e], %SW	18.4	18.5	18.6	0.59	0.87	18.6	18.4	0.67	1.53
Chilled carcass (CC), g	1695	1703	1682	0.68	0.60	1691	1694	0.90	142
Dressing out, %SW	61.5	61.1	61.2	0.37	0.45	61.2	61.3	0.58	1.63
Reference carcass (RC)[f], g	1429	1435	1417	0.69	0.64	1426	1428	0.92	127
Dissectible fat, %RC	3.16	3.35	3.33	0.44	0.61	3.27	3.29	0.94	1.01
Hind legs, %RC	33.0	32.7	32.7	0.44	0.55	32.9	32.6	0.31	1.00
Hind leg muscle/bone ratio	6.07	6.60	6.13	0.76	0.01	6.15	6.36	0.25	0.61

[a]Residual standard deviation. [b]Linear component of variance. [c]Quadratic component of variance. [d]Live weight immediately before slaughter; [e]Incidence of the full gastro-intestinal tract. [f]Chilled carcass without head, liver, thoracic organs and kidneys.

expense of insoluble fibre (Álvarez *et al*., 2007; Gómez-Conde *et al*., 2007). However, this was not our case, since villi height and villi/crypt ratio of jejunum decreased, which should be rather considered negatively (Table 4). On the other hand, the ileum mucosa morphology was not affected by dietary treatments, as also found in other studies of ours (Xiccato *et al*., 2008; Trocino *et al*., 2010, 2011).

The effect of the experimental factors was hardly measurable on slaughter results (Table 5), apart from a significant and quadratic change of the hind leg muscle to bone ratio (P=0.01) which indicates that diets with an intermediate (starch+soluble fibre)/ADF ratio are probably the most equilibrate and capable of stimulating the highest muscular growth of rabbits.

Effect of crude protein level – The decrease of dietary crude protein (CP) from 15% to 14% as-fed lowered CP digestibility (Table 2), likely because of the higher protein rate derived from alfalfa meal rather than soybean meal; besides, lower digestibility values were recorded for soluble fibre. Quite surprisingly, a limited decrement of dietary CP nearly halved the mortality rate (16.3% *vs*. 28.9%, P=0.01). On the other hand, it did not affect productive performance in the whole period, even if feed conversion tended to lower during the first period of trial and growth and feed intake decreased during the second period (Table 3). In fact, if 15% as-fed of dietary CP usually covers protein requirements for growing rabbits (de Blas and Mateos, 2010), the supply of main limiting amino acids (lysine, methionine and threonine) may permit a reduction until or below 14% (Trocino *et al*., 2000; García-Palomares *et al*., 2006). A higher dietary protein lowered caecal pH (5.87 to 5.70) (Table 4), whereas all microbial populations benefit from a high protein content in the caecum for their development and proliferation. However, some pathogenic strains of *E. coli* and *Clostridia* spp. may be at an advantage when there is an excess of protein (García-Palomares *et al*., 2006; Chamorro *et al*., 2007; Carabaño *et al*., 2009). In fact, Martínez-Vallespín *et al*. (2011) found that reducing dietary protein from 17.5 to 14.5% lowered mortality (−19.9 percentage points) of rabbits affected by ERE during post-weaning period (28-49 d of age), as we observed in the present trial.

Conclusions

On the basis of the results above, the contemporary increase of starch (until 20%) and soluble fibre (until 12%) had positive effects on digestive physiology, weight gain and feed efficiency of rabbits without impairing carcass traits or meat quality. However, in presence of epizootic rabbit enteropathy, the contemporary decrease of ADF below 13% might negatively affect digestive health of growing rabbits, by provoking a higher morbidity. Lowering the protein level from 15% to 14% had scarce effects on growth performance but reduced the susceptibility to digestive diseases.

References

Álvarez J.L., Margüenda I., García-Rebollar P., Carabaño R., De Blas J.C., Corujo A., García-Ruiz A.I. (2007). Effects of type and level of fibre on digestive physiology and performance in reproducing and growing rabbits. World Rabbit Sci. 15: 9-17.

Blas E., Gidenne T. (2010). Digestion of sugars and starch. In: Nutrition of the Rabbit, 2nd edition (C. de Blas, J. Wiseman, eds), CABI Publishing, Wallingford, UK, 19-38.

Blasco A., Ouhayoun J., Masoero G. (1993). Harmonization of criteria and terminology in rabbit meat research. World Rabbit Sci. 1: 3-10.

Carabaño R., García J., de Blas J.C. (2001). Effect of fibre source on ileal apparent digestibility of non-starch polysaccharides in rabbits. Anim. Sci. 72: 343-350.

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.

Chamorro S., Gómez-Conde M.S., Pérez de Rozas A.M., Badiola I., Carabaño R., De Blas J.C. (2007). Effect on digestion and performance of dietary protein content and of increased substitution of lucerne hay with soya-bean protein concentrate in starter diets for young rabbits. Animal 1: 651-659.

García J., Gidenne T., Falcao-e-Cunha L., de Blas C. (2002). Identification of the main factors that influence caecal fermentation traits in growing rabbits. Anim. Res. 51: 165-173.

García-Palomares J., Carabaño R., García-Rebollar P., de Blas J.C., Corujo A., García-Ruiz A.I. (2006). Effects of a dietary protein reduction and enzyme supplementation on growth performance in the fattening period. World Rabbit Sci. 14: 231-236.

Gómez-Conde M.S., García J., Chamorro S., Eiras P., Rebollar P.G., Pérez de Rozas A., Badiola I., de Blas C., Carabaño R. (2007). Neutral detergent-soluble fiber improves gut barrier function in twenty-five-day-old weaned rabbits. J. Anim. Sci. 85: 3313-3321.

Jehl N., Gidenne T. (1996). Replacement of starch by digestible fibre in feed for the growing rabbit. 2. Consequences for microbial activity in the caecum and on incidence of digestive disorders. Anim. Feed Sci. Technol. 61: 193-204.

Martínez-Vallespín B., Martínez-Paredes E., Ródenas L., Cervera C., Pascual J.J., Blas E. (2011). Combined feeding of rabbit female and young: Partial replacement of starch with acid detergent fibre or/and neutral detergent soluble fibre at two protein levels. Livest. Sci. 141: 155-165.

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., Falcao 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.

Trocino A., Xiccato G., Sartori A., Queaque P.I. (2000). Feeding plans at different protein levels: effects on growth performance, meat quality and nitrogen excretion in rabbits. World Rabbit Sci. 8 (suppl. 1): 467-474.

Trocino A., Fragkiadakis M., Radaelli G., Xiccato G. (2010). Effect of dietary soluble fibre level and protein source on growth, digestion, caecal activity and health of fattening rabbits. World Rabbit Sci. 18: 199-210.

Trocino A., Fragkiadakis M., Majolini D., Carabaño R., Xiccato G. (2011). Effect of the increase of dietary starch and soluble fibre on digestive efficiency and growth performance of meat rabbits. Anim. Feed Sci. Technol. 165: 265-277.

Trocino A., Fragkiadakis M., Majolini D., Tazzoli M., Radaelli G., Xiccato G. (2013a). Soluble fibre, starch and protein level in diets for growing rabbits: Effects on digestive efficiency and productive traits. Anim. Feed Sci. Technol. 180: 73-82.

Trocino A., García J., Carabaño R., Xiccato G. (2013b). A meta-analysis on the role of soluble fibre in diets for growing rabbits: a review. World Rabbit Sci. 21: 1-15.

Xiccato G., Trocino A., Carraro L., Fragkiadakis M. (2006). Digestible fibre to ADF ratio and protein concentration in diets for early-weaned rabbits. In: Proc 3rd Rabbit Congress of America, Maringá, Brasil, Comm. 35, pp. 1-6.

Xiccato G., Trocino A., Carraro L., Fragkiadakis M., Majolini D. (2008). Digestible fibre to starch ratio and antibiotic treatment time in growing rabbits affected by epizootic rabbit enteropathy. In: Proc 9th World Rabbit Congress, Verona, Italy, pp. 847-851.

acs78_39