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EFFECTS OF LEVELS OF INSOLUBLE AND SOLUBLE FIBRE IN DIETS FOR GROWING RABBITS ON FAECAL DIGESTIBILITY, NITROGEN RECYCLING AND *IN VITRO* FERMENTATION

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Abstract: The effect of neutral detergent fibre (NDF) and neutral detergent soluble fibre (NDSF) on *in vivo* faecal digestibility and caecal fermentation pattern was studied in growing rabbits, in 4 diets formulated according to 2 levels of NDF, 370 (LI) and 450 (HI) g/kg, and 2 levels of NDSF, 150 (LS) and 180 (HS) g/kg in substitution of starch, in a 2×2 factorial structure. Twenty four New Zealand White rabbits weaned at 28 d (630±80.2 g weight) were allocated to digestibility cages from 42 to 49 d of age to determine apparent faecal digestibility of each diet (n=6). Urine was collected for determination of purine derivatives (PD). Once the digestibility trial finished, rabbits were fitted with PVC neck collars for 24 h total caecotrophe collection. After 1 d of recovery, animals were slaughtered and caecal contents were used as inocula for 18 h *in vitro* gas production and caecal degradation (ivDMcD) study, using an HCl-pepsin and pancreatin pre-digested substrate. Diet digestibility was also determined by the *in vitro* three-step enzymatic procedure. There were no effects of the NDF×NDSF interaction for any digestibility parameter ($P>0.10$). Both dry matter and organic matter digestibility (DMd and OMd) decreased from 0.518 to 0.442 and from 0.526 to 0.447, respectively, with the increase of NDF ($P<0.001$), but were unaffected by the NDSF level ($P>0.10$). In contrast, NDF digestibility (NDFd) and ivDMcD did not respond to NDF ($P>0.10$) but increased from 0.156 to 0.200 and 0.141 to 0.210 with the proportion of NDSF ($P<0.01$). Weight of caecal contents increased with both NDF ($P<0.001$) and NDSF content ($P<0.01$). However, total production of caecotrophes increased from 20.1 to 25.5 g DM/d with NDF ($P<0.05$), but was not affected by NDSF. The crude protein (CP) proportion in caecotrophes decreased with NDF ($P<0.001$) and increased with NDSF level ($P<0.01$), and total CP recycled as caecotrophes tended ($P=0.093$) to be higher in HS diets, being unaffected by the dietary level of NDF. Diets rich in NDSF rendered higher gas volumes ($P<0.001$) than those with LS from 2 to 18 h incubation, whereas inclusion of high proportions of NDF reduced gas volume ($P<0.01$). Results indicate that NDF reduces faecal digestibility, whereas NDSF promotes better conditions for caecal fermentation.

Key Words: fibre level, soluble fibre, faecal digestibility, caecal fermentation.

INTRODUCTION

Compared with other non-ruminant species, the rabbit digestive system is characterised by the importance of the role of caecum. This organ contributes up to 0.40 of total digestive tract in weight, and harbours an abundant, diverse and highly active microbial community (Gouet

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and Fonty, 1979; Abecia *et al*., 2005; Monteils *et al*., 2008). As the animal matures, complex microbial interactions allow this organ to play a key part in rabbit digestive physiology as the major site for fermentation, but mainly for recycling of microbial protein through caecotrophy (Spreadbury 1978; Belenguer *et al*., 2002; Villamide *et al*., 2010).

Dietary fibre plays a dominant role in the regulation of the digesta flow, according to its level, source, size and composition (García *et al*., 2000; Gidenne and Bellier 2000). At the same time, the source of fibre helps determine the extent (Carabaño *et al*., 1988; Belenguer *et al*., 2002) and diversity (Gómez-Conde *et al*., 2009) of caecal microbial proliferation in the rabbit, thus contributing to the prevention of digestive pathologies (Gidenne, 2003).

The slowly fermentable dietary fibre is the most important factor regulating rate of passage and microbial activity (De Blas *et al*., 1999; García *et al*., 2000), decreasing mean retention time and thus favouring caecal turnover, although at the same time its low fermentability would contribute to impairing the rate of microbial protein synthesis (García *et al*., 2000). However, this response largely depends on both the amount of fibre and its degree of lignification. On the other hand, easily fermentable fibre (neutral detergent soluble fibre, NDSF), which is partially fermented in the small intestine (Gidenne, 1992; Carabaño *et al*., 2001), should stimulate caecal fermentation and microbial nitrogen recycling through an increase in caecal weight when included in moderate proportions (Gidenne and Bellier, 2000; García *et al*., 2000; Gidenne *et al*., 2004; Gómez-Conde *et al*., 2009).

Interaction between proportions of slowly and highly fermentable fibre fractions in rabbit caecal fermentation creates a complex scenario where the overall digestive processes have not yet been clarified. This work studied the interaction between two levels of insoluble dietary fibre (neutral detergent fibre, NDF) and two of neutral detergent soluble fibre (NDSF) on apparent digestibility and dietary parameters describing caecal fermentation patterns in growing rabbits.

MATERIALS AND METHODS

Diets

Four experimental diets, including 2 different levels of NDF (low, LI and high, HI) and 2 levels of NDSF (low, LS and high, HS) for each NDF level, were assayed in a 2×2 factorial structure, thus resulting in LILS, LIHS, HILS and HIHS diets. The amount of NDSF in each diet was included by replacing an equivalent proportion of starch. Diets were formulated on an isoprotein basis, including 145 g crude protein (CP) per kg dry matter (DM). Ingredients and chemical composition of diets are presented in Table 1. Zinc bacitracin (Bacipremix 50, Andrés Pintaluba SA, Reus, Spain) and robenidine (Cycostat 66G, Alpharma Inc, USA) were included in the feeds to prevent pathogens proliferation. Feeds were given pelleted to 3.0 mm diameter and offered *ad libitum*. Water was freely available throughout the experimental period.

Animals

Twenty-four New Zealand White rabbits weighing 630±80.2 g were weaned at 28 d of age and used experimentally. Due to the limited availability of digestibility cages, this study was conducted in 3 experimental periods. In each period, 4 rabbits of similar weights from each of 2 litters (n=8) were chosen and allocated to the 4 experimental treatments, resulting in 2 rabbits for different litters per diet in each period. All procedures were carried out under Project Licence PI27/08 approved by the Ethical Advisory Committee on Animal Experiments from the

Table 1: Ingredients (g/kg) and chemical composition (g/kg dry matter (DM)) of experimental diets[1].

	LILS	LIHS	HILS	HIHS
Ingredient				
Wheat grain	180	90	90	---
Wheat bran	40	40	---	---
Sugarbeet pulp	100	270	100	270
Oat hulls	40	90	100	150
Soybean hulls	40	---	40	---
Grape marc	180	170	240	230
Cereal straw	210	130	208	128
Soybean protein concentrate	75	75	75	75
LT Fishmeal	20	20	22	22
Maize starch	---	---	10	10
Sugarcane molasses	10	10	10	10
Soybean oil	50	50	50	50
L-lysine	5.75	5	5.75	5
DL-methionine	4	4	4.25	4.25
L-threonine	3.5	3.25	3.75	3.5
L-tryptophan	2.25	2.25	2.25	2.25
Calcium carbonate	3	3	---	---
Dicalcium phosphate	27.5	28.5	30	31
Sodium chloride	5	5	5	5
Vitamin-mineral premix[2]	4	4	4	4
Chemical composition				
Organic matter	918	919	920	920
Crude protein	145	145	152	144
Ether extract	85	81	73	71
Neutral detergent fibre	370	384	456	460
Acid detergent fibre	224	229	293	288
Lignin	60	59	117	105
Neutral detergent soluble fibre	148	186	154	180
Starch	138	100	87	45
Purine bases (mmol/kg DM)[3]	6.07	5.79	4.88	4.69
Digestible energy (kcal/kg DM)[4]	2391	2394	2092	2095

[1]Bacipremix 50 (zinc bacitracin), 100 mg/kg and Cycostat (robenidine hydrochloride), 66 mg/kg were added.
[2]TEGASA (Barcelona, Spain). Declared composition (g/kg): 3,75: Cu ($CuSo_4$); 25: Zn (ZnO); 0.0063: Se (Na_2O_3Se); 0.063: I (KI); 5: Mn (MnO); 0.025: Co ($CoSO_4$ $7H_2O$); 10: Fe (FeCO3); 1875000 UI: Vitamin A; 250000 UI: Vitamin D3; 3.75: α-tocopherol; 0.1: Vitamin K3; 0.2: Thiamine; 0.25: Riboflavin; 0.2: Pyridoxine; 1250: Cyanocobalamin; 3: Nicotinic acid; 1.25: Pantothenic acid; 37.5: Choline chloride.
[3]Estimated from ingredient composition according to Pérez *et al*. (1996).
[4]Estimated according to FEDNA (2003).

University of Zaragoza, Spain. Care and use of animals were performed according to the Spanish Policy for Animal Protection RD1201/05, in compliance with European Union Directive 86/609 on the protection of animals used for experimental and other scientific purposes.

Rabbits were given the experimental diets for 14 d in a controlled environment room (18-24ºC, 08 a.m. to 08:00 p.m. light schedule) and then transferred to individual metabolism cages (40×50×45 cm). After 2 d of adaptation to the cages, feed refusals, faeces and urine excretion were monitored daily for 5 d. Faeces were weighed and immediately frozen (-20ºC) for further analyses. Urine was collected individually over 50 mL of 1 M H_2SO_4 (to keep final pH below 3) and weighed. Then, the whole urine was diluted to 1 L with distilled water, and 50 mL sampled and stored at −20ºC for determination of urinary purine derivatives (PD). Animals were weighed individually at the start of the experimental period (28 d of age) and at the beginning (42 d) and end (49 d) of the digestibility trial. In addition, *in vitro* digestibility was also determined enzymatically by the 3 step method of Boisen and Fernandez (1997).

The day after the digestibility trial finished, PVC neck collars (6 cm inner diameter and 27 cm outer diameter) were fitted at 7:00 a.m. to the rabbits for 24 h for total collection of soft faeces (caecotrophes). Animals were allowed to recover for 1 d and then slaughtered by cervical dislocation between 11:00 a.m. and 12:00 p.m. for *in vitro* caecal fermentation studies. Their caeca were excised, weighed and emptied into a sterile container. The pH of caecal contents was measured directly inside the organ with a glass electrode pH-meter (CRISON 507, CRISON, Barcelona, Spain). Caecal contents were immediately used as fermentation inocula for *in vitro* gas production study.

In vitro gas production

The microbial fermentation pattern of caecal contents was studied *in vitro* by the gas production technique (Theodorou *et al*., 1994). The incubation solution did not include resazurin or microminerals, as suggested by Mould *et al*. (2005), and incubation pH was adjusted to 6.5 as described in Fondevila and Pérez-Espés (2008). A 0.8 g amount of pre-digested feeds by the first 2 steps (HCl-pepsin and pancreatin incubation) of the enzymatic digestibility procedure of Boisen and Fernández (1997) was used as substrate, to simulate the digestive processes occurring before the caecum. Duplicate glass bottles (116 mL total volume) were filled with 72 mL incubation solution and incubated with caecal contents of each of the 6 rabbits for each treatment (8 g, which implies a 0.10 of total incubation solution, assuming a density of 1). In addition, duplicate bottles without substrate were also included as blanks for each inoculum. Bottles were sealed under CO_2 stream and incubated at 39ºC for 18 h. Gas pressure produced in each bottle was recorded with a HD8804 manometer with a TP804 pressure gauge (DELTA OHM, Caselle di Selvazzano, Italy) after 2, 4, 6, 8, 10, 12 and 18 h incubation. Incubation was halted at 18 h by dipping the bottles in cold water, then their content was filtered through pre-weighed nylon bags (45 mm pore size) and dried at 60ºC for 48 h to estimate the *in vitro* DM caecal degradation (ivDMcD). Readings were converted to volume by using a pre-established linear regression between pressure recorded in the same type of bottles and known inoculated air volumes (Marinas *et al*., 2003). Gas volume at each incubation time was expressed per gram of incubated DM.

Chemical analyses

Chemical analyses of feeds were carried out following the AOAC (2005) procedures relating to dry matter (DM; 934.01), organic matter (OM; 942.05), crude protein (CP; 976.05) and ether extract (EE; 2003.05) analyses. Concentrations of neutral and acid detergent fibre (NDF and

ADF) and lignin were determined sequentially according to Van Soest *et al*. (1991) using an Ankom 220 Fibre Analyser equipment (Ankom Technology, New York). NDF and ADF are expressed excluding residual ash, and an α–amylase was used in the NDF analysis. Sodium sulphite was not used. Proportion of NDSF in feeds was estimated according to Hall *et al*. (1997). Starch was determined by polarimetry after hydrolysis of feeds with HCl (European Communities Commission, 1999).

Samples of experimental diets were successively digested with HCl-pepsin (Sigma P-7000), pancreatin (Sigma P-1750) and viscozyme (Novo-Nordisk, Denmark) solutions, following the Boisen and Fernandez method (1997) to determine the *in vitro* total and ileal DM digestibility by using the 3 or 2 steps methodology (ivDMd3 or ivDMd2, respectively). Urinary PD (allantoin and uric acid) were analysed by reverse-phase HPLC, using 2 Spherisorb C-18 ODS-2 (4.6×250 mm) columns, according to the technique described by Balcells *et al*. (1992).

Statistical analyses

Data were analysed in a 2×2 factorial structure, considering the experimental period as block, using the General Lineal Model (GLM) procedure from SAS (2008). Gas production results were analysed within each incubation time. Means from the interaction of treatments were compared by the least significant difference test, and differences among means with $P<0.05$ and $0.05<P<0.10$ were accepted as statistically significant or close to significance, respectively.

RESULTS

All animals remained in good health throughout the experimental period. They adapted well to the experimental diets and neither morbidity nor mortality problems were observed. No treatment differences ($P>0.10$) were observed in rabbit weight at the end of the experimental period, corresponding to 49 d of age (average weight of 1488 g), and no differences in growth rate were recorded (Table 2). Daily feed intake throughout the digestibility trial increased ($P<0.001$) with the amount of dietary NDF, but was unaffected by NDSF.

Coefficients of *in vivo* faecal dry matter, organic matter and neutral detergent fibre digestibility (DMd, OMd and NDFd), ivDMd2, ivDMd3 and ivDMcD are shown in Table 2. Considering the lack of sample replicates, the effect of the studied factors on ivDMd was not statistically contrasted. The effect of the NDF×NDSF interaction was not significant for any digestibility coefficient ($P>0.10$). Both DMd and OMd decreased by 14-15% with the level of dietary inclusion of NDF ($P<0.001$), but were unaffected by the level of NDSF ($P>0.10$). The ivDMd3 agreed well with DMd (r=0.988; $P=0.008$; n=4), although observed values were 6 to 8 percentage units higher. Considering only the first 2 steps of the procedure (ivDMd2), precaecal digestibility accounted for 0.65 to 0.77 of total digestibility in rabbits fed HS and LS diets, respectively. In contrast, NDFd was not affected by the level of NDF ($P>0.10$), but increased from 0.14 to 0.21 with the proportion of NDSF ($P<0.01$). This response agrees with the higher ivDMcD of HS diets compared to LS diets, which was positively correlated with NDFd (r=0.420; $P=0.041$; n=24).

No differences were observed in caecal pH (Table 3). Relative weight of caecal contents increased in 18% with the level of NDF ($P<0.001$), but also increased 16% with the NDSF proportion ($P<0.01$). Soft faeces excretion increased by 20% in diets with the higher level of NDF ($P<0.05$). However, since CP proportion decreased with NDF ($P<0.001$) and increased with NDSF ($P<0.01$), total CP output tended ($P=0.093$) to be higher in HS diets, as soft faeces

Table 2: Dry matter intake, growth rate, coefficients of *in vivo* faecal digestibility, *in vitro* dry matter degradation and *in vitro* dry matter digestibility in caecal contents (ivDMd) in growing rabbits given diets with low (LI) or high (HI) levels of NDF and low (LS) or high (HS) levels of NDSF (n=6).

						P-value	
	LILS	LIHS	HILS	HIHS	SD	NDF	NDSF
DM intake (g/d)	114.2	113.5	139.3	135.3	8.3	<0.001	NS
Growth rate (g/d)	27.14	31.14	26.29	28.91	4.85	NS	NS
In vivo faecal digestibility							
Dry matter	0.512	0.524	0.439	0.444	0.016	<0.001	NS
Organic matter	0.521	0.531	0.446	0.448	0.016	<0.001	NS
Neutral detergent fibre	0.152	0.215	0.159	0.185	0.032	NS	0.003
In vitro caecal degradation (ivDMcD)	0.141	0.225	0.140	0.195	0.057	NS	0.008
In vitro digestibility							
2 steps (ileal. ivDMd2)	0.464	0.405	0.379	0.330			
3 steps (faecal. ivDMd3)	0.588	0.604	0.500	0.521			
Caecal (ivDMd3–ivDMd2)	0.123	0.199	0.122	0.191			

P>0.10 for NDF×NDSF interaction; NS: *P*>0.10. SD: standard deviaton.

was unaffected by the dietary level of NDF. No treatment effects were observed when the production of caecotrophes was related to caecal content weight as an index of caecal turnover through caecotrophy, being on average 1.52.

Among the PD excreted in urine, xanthine and hypoxanthine were only detected as tracer amounts, and therefore data are not presented and only allantoin and uric acid were considered in the estimation of total PD excretion. No differences in allantoin excretion were recorded among those rabbits receiving the low NDF level (LI diets); however, among rabbits fed the highest proportion of NDF (HI), those given the high NDSF level excreted less allantoin (diet HIHS *vs*. HILS; NDF×NDSFb interaction; $P=0.069$). This result close to the significance reached significance in urinary excretion of uric acid ($P<0.05$), and consequently total urinary excretion of PD was lower in animals given the HIHS diet ($P<0.05$).

The gas production pattern from the 4 predigested diets is presented in Figure 1, expressed as gas volume per g DM of incubated substrate. For each incubation time, inclusion of the highest proportion of NDF reduced gas volume ($P<0.01$), whereas diets rich in NDSF rendered higher gas volumes ($P<0.001$) than those with LS from 2 to 18 h incubation. Again, no significant effects of the interaction NDF×NDSF were detected at any incubation time ($P>0.10$). The volume of gas produced after 18 h of *in vitro* incubation with caecal contents was correlated with ivDMcD (r=0.450; $P=0.029$; n=24) and NDFd (r=0.563; $P=0.007$; n=24).

DISCUSSION

In this work, 2 different *in vitro* procedures were applied to study overall diet digestibility in rabbits. Firstly, the enzymatic digestion procedure (Boisen and Fernandez, 1997) simulates the digestion processes occurring at gastric, small intestine and hindgut levels. Estimated digestibility values have been shown to be highly correlated with *in vivo* faecal dry matter digestibility (Ramos *et al*., 1992; Pascual *et al*., 2000; Villamide *et al*., 2009). In our case, even though the

Table 3: Caecal pH, caecum weight, daily production of caecotrophes and urinary excretion of purine derivatives (PD, µmol/d) as estimation of microbial protein recycling in growing rabbits given diets with low (LI) or high (HI) levels of NDF and low (LS) or high (HS) levels of neutral detergent soluble fibre (NDSF) (n=6).

						P-value		
	LILS	LIHS	HILS	HIHS	SD	NDF	NDSF	NDF×NDSF
Caecal pH	6.32	6.11	6.21	6.16	0.24	NS	NS	NS
Caecum weight (g):								
total weight	86.9	92.4	100.4	112.6	8.8	<0.001	0.023	NS
empty organ	28.1	27.4	30.0	29.0	2.0	0.051	NS	NS
content[1]	3.85	4.32	4.52	5.42	0.51	<0.001	0.01	NS
Caecotrophes:								
g DM/d[1]	1.31	1.43	1.64	1.78	0.34	0.024	NS	NS
CP (g/g DM)	0.241	0.253	0.192	0.219	0.930	<0.001	0.002	NS
g CP/d	4.63	5.33	4.71	5.75	1.20	NS	0.093	NS
g DM caecotrophes/ g DM caecal contents	1.58	1.42	1.67	1.39	0.08	NS	NS	NS
PD excretion (µmol/d):								
Allantoin	699.2	766.7	824.1	595.5	199.6	NS	NS	0.067
Uric Acid	195.7[a]	201.8[a]	194.2[a]	167.6[b]	14.6	0.01	NS	0.016
Total PD	864.9[ab]	968.5[ab]	1018.3[a]	762.1[b]	201.9	NS	NS	0.049

[1]g/100 g body weight; NS: $P>0.10$. Within rows, different letters indicate significant differences among means ($P<0.05$)
SD: standard deviation. DM: dry matter. CP: crude protein.

scarcity of data does not allow the establishment of a linear relationship, results of ivDMd3 fitted well with those of DMd. The fact that estimates were 6 to 8 percentage units over that of DMd is in agreement with Pascual *et al*. (2000) and Villamide *et al*. (2009) and would be at least partly explained by the lack of an endogenous contribution *in vitro*, although some degree of losses when filtering cannot be ruled out. However, even though cellulolytic enzymes have the potential to simulate digestion in the hindgut, the fermentation process as a whole includes the taking up of degraded products from digestive breakdown and their utilisation by living microbes, thus releasing gaseous and volatile by-products. In this way, the impact of caecal fermentation on the overall digestion process, especially that of fibre, was approached by the gas production technique (Theodorou *et al*., 1994), which has previously been applied to rabbits (Calabrò *et al*., 1999; Chang *et al*., 2007). However, translation of this technique to hindgut fermentors requires the consideration of previous digestive processes, and therefore the use of pre-digested feeds as substrates (Fondevila *et al*., 2002; Bauer *et al*., 2003).

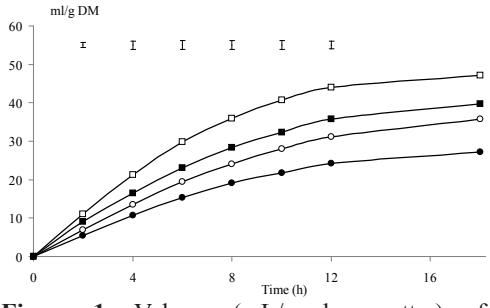

Figure 1: Volume (mL/g dry matter) of gas produced when pre-digested diets were incubated with caecal contents (n=6). (–□–) LIHS diet, (–■–) HIHS diet, (–○–) LILS diet and (–●–) HILS diet (abbreviations as in Table 3). Upper bars represent standard error of means.

As expected, faecal digestibility (DMd, OMd) was reduced in HI diets. However, this occurred despite the level of NDSF ($P>0.10$), probably because soluble fibre in HS diets partly substitutes another component of similar digestibility, such as starch. It is difficult to verify this speculation, but NDSF can be partly digested in the small intestine and the remaining fraction is rapidly and extensively fermented in the rabbit caecum (Gidenne 1992; García *et al*., 1999; Carabaño *et al*., 2001).

Within the NDF ranges of experimental diets (from 37 to 46% NDF), the higher caecal contents in HI diets (Table 2) were expectable (de Blas *et al*., 1999), and might be associated with a higher feed intake as NDF increases (r=0.664; $P<0.001$; n=24). However, this was not associated with an increase in NDFd, probably because of the higher degree of lignifications of NDF in HI diets. Despite the dietary NDF content, the higher faecal NDFd recorded in HS diets is related to a stimulation of the fermentative processes in the caecum promoted by the pectin constituents of the NDSF of sugarbeet pulp. An increase in caecal content weight ($P<0.001$) is also promoted by NDSF, as was previously observed with the inclusion of sugarbeet pulp (García *et al*., 1993; Carabaño *et al*., 1997; Belenguer *et al*., 2002). It is worth mentioning that the main goal of the rabbit caecum is to ferment the easiest available substrate (soluble or low sized particles) for microbial synthesis and protein recycling rather than for fibre digestion itself (Balcells *et al*., 1998; Belenguer *et al*., 2005; Villamide *et al*., 2010). This would explain the low magnitude of NDFd, as NDF is mostly excreted in hard faeces, and the reduction of crude protein digestibility when caecotrophy is not allowed (Villamide *et al*., 2010).

The increased amount of caecal contents with NDF was reflected in a higher total excretion of caecotrophes in HI diets, but no response to NDSF was observed in this despite the differences in caecal content weight. The lower fermentative activity (in terms of gas production through the 18 h incubation period) observed in diets with HI was highly correlated (r=0.726; $P<0.001$; n = 24) with the crude protein content of caecotrophes that might be considered as an index of microbial biomass production, and this was higher in HS diets as it promotes a higher microbial activity (García *et al*., 2000). This resulted in a trend ($P=0.093$) towards a higher crude protein recycling through caecotrophes in high NDSF diets. However, these results do not agree with the results of urinary excretion of total PD, which showed that when diets are high in NDF, total PD decreases with NDSF ($P<0.05$). In this sense, it is worth noting that urinary PD excretion reflects intake of purine bases from both microbial, from soft faeces, and dietary origin (Balcells *et al*. 1998). Estimated dietary intake of purine bases (PB; Table 1) was similar among diets (0.693, 0.657, 0.680 and 0.635 mmol/d for LILS, LIHS, HILS and HIHS, respectively), and microbial purine intake should accordingly follow the same pattern as urinary PD excretion.

In the PD method (Balcells *et al*., 1998; Belenguer *et al*., 2008), intake of microbial purines bases is estimated assuming a urinary recovery of ingested PB of 0.67 and considering that microbial plus dietary purines make up total ingested PB. On this basis, microbial PB intake could be calculated by difference (from total PD excretion and that derived from diet intake) as 0.60, 0.79, 0.84 and 0.50 mmol/d for LILS, LIHS, HILS and HIHS, respectively. Moreover, as dietary PB are either absorbed or used by caecal microorganisms, then faecal PB are of microbial origin and therefore can be used as a microbial marker. If a constant microbial N to PB ratio in caecal contents is assumed (1.3 mmol PB/g N; Belenguer *et al*., 2008) then microbial CP recycling through caecotrophy would be 2.88, 3.79, 4.04 and 2.41 g/d, for LILS, LIHS, HILS and HIHS, respectively. Thus, values of microbial CP recycling behave similarly to total urinary PD excretion, and with high NDF diets those rabbits fed the highest level of soluble fibre

(HIHS) showed a low level of microbial CP recycling, in contrast with the *in vivo* and *in vitro* fermentation results.

Total CP excretion through caecotrophes (Table 3) was higher and showed a different pattern than the estimation of CP recycling by the PD method. Although most of the N in caecotrophes is of microbial origin, a non-microbial N fraction coming from dietary and distal colon endogenous contribution is also apparent.

The higher ivDMcD and *in vitro* gas production with HS compared to LS diets indicated the role of soluble fibre enhancing caecal fermentation, in agreement with *in vivo* studies (Carabaño *et al*., 1997; Gidenne and Bellier, 2000; Gómez-Conde *et al*., 2009). Moreover, estimation of caecal digestion through the third step of the enzymatic procedure from Boisen and Fernandez (1997) (ivDMd3 – ivDMd2) gives similar results to those from ivDMcD (r= 0.964; *P*= 0.024; n= 4). Neutral detergent soluble fibre as a source of easily fermentable carbohydrates would promote growth and activity of caecal microbiota, behaving as a powerful microbial activator. The lack of response to NDSF in caecal pH is in agreement with Gómez-Conde *et al*. (2009), probably because caecal fermentative activity was low at the time of slaughter (Bellier and Gidenne, 1996).

CONCLUSIONS

Dietary fibre has a direct effect on the efficiency of the rabbit digestive tract which results in a decrease in dry matter and organic matter digestibility as NDF increases, this effect being independent of the NDSF content. Soluble fibre improves caecal fermentative and microbial activity, thus increasing NDF digestibility.

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