EFFECT OF FIBRE SOURCE ON NEUTRAL DETERGENT FIBRE DIGESTION AND CAECAL TRAITS IN RABBITS

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Abstract - Sixty New Zealand White x Californian rabbits were used to evaluate apparent neutral detergent fibre digestibility (NDFd) and caecal traits (soft faeces excretion, weight of caecal contents (DCC, % body weight), pH and VFA (mmol l^{-1}) and ammonia concentrations ($N\text{-}NH_3$, mmol l^{-1})) of six fibrous sources: paprika meal, olive leaves, lucerne hay, soya-bean hulls, sunflower hulls and NaOH-treated barley straw. Six diets were formulated by supplementing the fibrous feeds with a concentrate free of fibre containing starch, protein, fat and a min/vit mix. Source of fibre affected significantly ($P<0.001$) all the variables measured. NDFd ranged from 3.0 to 35.1%. A stepwise regression analysis was used to obtain a prediction equation of NDFd from chemical composition, particle size (FP: Proportion of fine particles (<0.315 mm); LP: Proportion of large particles, (>1.25 mm)) and caecal traits (n=60): NDFd= 32.93(±3.01) + 0.29(±0.15) ($N\text{-}NH_3$) - 1.27 (±0.23) Lignin - 1.83(±0.27) LP (R^2=0.627; $P<0.001$). Caecal pH ranged from 5.61 to 6.28 among diets. Two equations, using chemical composition and particle size of feeds or caecal traits as independent variables, were calculated to predict caecal pH by stepwise regression analysis (n=58): pH= 6.72 (±0.11) - 0.067(±0.011) Uronic Acids - 0.0074(±0.0012) FP (R^2=0.533; $P<0.001$), and pH= 6.76 (±0.14) + 0.0072(±0.0036) ($N\text{-}NH_3$) - 0.011(±0.0021) VFA -0.38(±0.060) DCC (R^2=0.573; $P<0.001$).

INTRODUCTION

Fibre is one of the main nutrients of rabbit diets which usually contain a 35-40% of NDF. Lucerne hay is the source of fibre preferred in rabbit diets and its substitution with other alternative fibre sources (sugar-beet pulp and grape marc), differing in chemical composition and physical structure, affects significantly the digestion in rabbits (MOTTA, 1990; FRAGA *et al*, 1991).

The aim of this work was to compare the effect of six fibrous sources: paprika meal, olive leaves, lucerne hay, soya-bean hulls, sunflower hulls and NaOH-treated barley straw on fibre digestion. These feeds show a wide range of variation in their chemical composition (NDF content and degree of lignification of NDF) and particle size.

MATERIAL AND METHODS

Diets

Six diets were formulated to contain as sole source of fibre paprika meal, olive leaves, lucerne hay, soya-bean hulls, sunflower hulls or NaOH-treated barley straw. Fibre sources were supplemented with different proportions of soya protein isolate (ARDEX-AF. Loders Croklaan), wheat flour, lard, minerals, DL-methionine and a min/vit mix, to obtain balanced diets containing at least 18.5% CP and 10% starch. Ingredient and chemical and physical analyses of diets and fibre sources are shown in Table 1.

Digestibility Trial

A group of 60 male and female New Zealand White x Californian rabbits (ten per diet), from 48 to 54 days of age, and weighing from 1.3 to 1.5 kg, were randomly allotted to the six diets. Animals were fed *ad libitum*. Following a 14-d period of adaptation to each diet, intake was recorded and collections were made on 4 consecutive days. Faeces produced daily were stored in polyethylene bags at -20°C. Faeces were dried and analysed for NDF.

Caecal Trial

Two days after finishing the digestibility trial, a wooden collar (150 g and 25 cm diameter) was put on each animal to prevent caecotrophy. The collar was put on at 8.00 h, half an hour after light was switched on, and was removed 24 h later. The soft faeces collected were stored at -20° C. Feed intake was recorded for three days before the collar was placed and animal were weighed just before the collar was placed. Seven days later the same procedure was repeated. Soft faeces were analyzed for DM.

Table 1 : Ingredient and chemical composition of diets. Chemical composition and particle size of fibrous sources

Item	Paprika meal	Olive leaves	Lucerne hay	Soya-bean hulls	NaOH-treated straw	Sunflower hulls
Ingredient, %						
Paprika meal	75.0	--	--	--	--	--
Olive leaves	--	75.0	--	--	--	--
Lucerne hay	--	--	75.0	--	--	--
Soya-bean hulls	--	--	--	62.0	--	--
Treated straw	--	--	--	--	62.0	--
Sunflower hulls	--	--	--	--	--	62.0
Wheat flour	15.4	7.8	15.4	20.8	18.3	18.3
Soya protein isolate	5.0	12.6	5.0	12.0	15.5	15.5
Lard	1.25	1.25	1.25	1.25	1.25	1.25
DL-methionine	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
Calcium carbonate	0.60	0.60	0.60	0.90	0.50	0.50
Calcium phosphate	1.95	1.95	1.95	2.25	1.65	1.65
Min/vit mix[a]	0.20	0.20	0.20	0.20	0.20	0.20
Chemical analysis of experimental diets, % DM						
CP	20.5	18.9	20.3	22.7	19.5	20.8
NDF	25.5	28.5	31.6	34.8	42.5	47.2
Starch	13.9	10.2	14.5	21.0	16.9	16.8
Chemical analysis of fibrous sources, % DM						
NDF	33.1	38.2	40.8	56.8	70.5	78.7
ADF	30.9	30.6	31.5	41.8	44.9	62.6
ADL	17.2	16.3	7.0	2.2	6.0	22.8
ADC	10.0	6.8	2.2	0.8	2.0	8.1
UA	5.2	5.6	7.9	9.1	1.9	8.0
CP	19.0	8.7	17.0	13.9	3.7	4.8
CP-NDF	2.6	3.9	4.7	4.4	1.9	2.7
FP	93.2	62.2	71.5	46.9	46.4	26.2
LP	0.0	9.4	2.1	3.7	10.9	4.5

[a] Provided by Colborn Dawes SA. Mineral and vitamin composition (g kg^{-1}): Mn, 13.4; Zn, 40; I, 0.7; Fe, 24; Cu, 4; Co, 0.35; riboflavin, 2.1; calcium panthothenate, 7.3; nicotinic acid 18.7; vitamin K_3, 0.65; vitamin E, 17; thiamine, 0.67; pyridoxine, 0.46; biotin, 0.04; folic acid, 0.1; vitamin B_{12}, 7 mg kg^{-1}; vitamin A, 6,700.000 IU kg^{-1}; vitamin D_3, 940,000 IU kg^{-1}.
NDF:Neutral detergent fibre. ADF:Acid detergent fibre. ADL:Acid detergent lignin. ADC:Acid detergent cutin. UA:Uronic Acid. CP:Crude protein.CP-NDF: Neutral detergent insoluble protein. FP: Fine particles (<0.315mm). LP:Large particles (>1.25mm)

Seven days after the last caecotrophy control, the animals were slaughtered by cervical dislocation one hour before dark (at 18.30 h), to avoid the caecotrophy period. They weighed 2346±647(SD) g on average. The caecal content was removed, its pH measured, and it was divided into two samples. One was used to determine DM. The other sample was centrifuged at 15000 rpm at 0° C for 10 min. The supernatant fluid was used to determine ammonia concentration and total concentration of VFA. A solution of 5% orthophosphoric acid (v/v) plus 1% mercury chloride (w/v) was added (0.1 ml ml^{-1}) to the samples for VFA determination. Samples for ammonia determination were acidified with a solution 0.2M hydrochloric acid (1 ml ml^{-1}).

Housing

The animals were housed in metabolism wired cages (405 x 510 x 320 mm high) that allowed separation of faeces and urine. The rabbits were kept in a closed building with partial environmental control, under a 12-12 h light-dark schedule. The light was switched on at 7.30 h. Temperature during the experimental period ranged from 16 to 24°C.

Analytical Methods

Chemical analysis were performed using the method of Van Soest *et al.* (1991) for NDF and Goering and Van Soest (1970) for ADF, ADL, acid detergent cutin (ADC) and crude protein on NDF residue (CP-NDF). Neutral detergent fibre was determined directly, whereas ADF and ADL were extracted successively. Cutin was determined after extracting successively ADF, ADL and permanganate lignin. Procedures of AOAC (1984) were used for DM and CP. Uronic acids (UA) were determined by the m-phenyphenol method (BLUMENKRANTZ and ASBOE-HANSEN, 1973).

Proportion of fine and large particles (FP:<0.315 mm, LP:>1.25 mm, respectively) in feeds were determined on two samples of pellets by wet sieving. A dried sample of 55 g was placed in 1100 ml distilled water and 10 ml commercial detergent. It was left overnight stirring. Then it was emptied onto a sieve stack (Filtra 4/1. Barcelona) with five sieves with decreasing pore sizes: 2.5, 1.25, 0.635, 0.315 and 0.160 mm, and washed with water for 5 min. The 2.5 mm sieve was then removed, allowed to drain for 1 hour and weighed. The same process was done during 15, 10, 6, and 4 min for the sieves with pore sizes 1.25, 0.635, 0.315 and 0.160 mm, respectively. The flow of water used was 1,5-2 l min^1. After that the sample collected was transferred to a tray and DM determined.

Caecal ammonia ($N-NH_3$) was analyzed using the autoevaluation distillation unit Kilab nitrolab-auto. Samples were distilled with a solution of sodium tetraborate (2.5%), collected on boric acid solution (1%) and valorated with hydrochloric acid (0.005 M) and a colour indicator. Caecal volatile fatty acid (VFA) concentration was determined in a Hewlett-Packard (5710 A) gas chromatograph, as it is described by GARCIA *et al* (1995).

Statistical analysis

Statistical analyses were performed using the GLM procedure of SAS (1985). Stepwise regression analysis was employed for developing prediction equations. The stepwise procedure introduced variables into the model only if they contributed to a significant improvement (P<0.15) in the estimation of the dependent variables.

RESULTS AND DISCUSSION

NDF digestibility

Source of fibre influenced (P<0.001) NDF digestibility (NDFd, %) (Table 2).

Table 2 : Effect of fibre source on NDF digestibility and caecal digestion traits.

	Paprika meal	Soya bean hulls	Lucerne hay	NaOH treated straw	Sun-flower hulls	Olive leaves	SEM[1]	P[2]
NDFd[3], %	35.1	28.2	17.5	16.7	10.0	3.0	1.81	<0.001
Caecal traits[4]:								
Soft faeces excretion, gDM 100^{-1} g^{-1} BW d^{-1}.	1.39	0.94	1.01	0.80	0.86	1.43	0.08	<0.001
Caecal dry matter, %	24.7	26.5	21.7	19.7	24.5	25.9	0.58	<0.001
Dry caecal contents, %BW	1.51	1.28	0.87	0.76	0.84	1.39	0.09	<0.001
pH	5.68	5.61	5.83	6.28	6.09	5.73	0.05	<0.001
VFA, mmol l^{-1}	45.4	64.4	64.3	38.2	49.5	45.2	2.01	<0.001
$N-NH_3$,mmol l^{-1}	15.0	11.8	9.6	18.0	16.5	2.4	1.43	<0.001

[1]SEM: Standard error of means (n=10). [2] Probability of significant differences among treatment means. [3]NDFd: Apparent NDF digestibility.[4]For caecal traits of NaOH-treated straw n=8.

A stepwise regression analysis was done for predicting NDFd (%) of feeds from their chemical composition, particle size and caecal traits. The results obtained were (n=60):

Step 1: NDFd=28.27(±2.04) - 2.00(±0.33)LP
R^2=0.398 P<0.001

Step 2: NDFd=36.73(±2.31) - 1.26(±0.24) Lignin - 1.92(±0.27)LP
R^2=0.602 P<0.001

Step 3: NDFd=32.93(±3.01) + 0.29(±0.15)($N-NH_3$) - 1.27(±0.23)Lignin - 1.83(±0.27) LP,
R^2=0.627 P<0.001

where LP was proportion of particles larger than 1.25 mm, lignin was calculated as ADL-ADC (Table 1) and $N-NH_3$ was the ammonia concentration in the caecum (mmol l^{-1}) (Table 2).

The effect of proportion of large particles (LP:>1.25 mm) might be explained by a lower caecal fermentation time. Lignin (ADL-cutin) difficult NDF digestion through its covalent linkages with carbohydrates. A minimum of caecal ammonia concentration seemed to be required to allow a normal microbial activity.
Paprika meal showed the highest NDF digestibility (35.1%), although its relatively high cutin and lignin content (10.0 and 7.2%). This result might be explained by its high proportion of fine particles (93.2%) that would increase the caecal retention time according to GIDENNE (1993), and it is in agreement with the relatively high values obtained for weight of caecal contents with this source of fibre (1.51%).
Soya-bean hulls NDF was rather digestible (28.2%), probably due to its low lignin content (1.4%). However it was lower than for paprika meal, which could be related to its lesser proportion of fine particles (46.9%).
NDFd of NaOH-treated straw was similar from that of lucerne hay (16.7 vs 17.5%). NaOH-treated straw had a larger particle size, but a slightly lower lignin content than lucerne hay (4.0 vs 4.8%). The treatment with NaOH breaks down the covalent linkages of lignin with carbohydrates, which favours NDF digestion (VAN SOEST, 1993).
Digestibility of sunflower hulls NDF was low (10.0%), which is in accordance with its high lignin content (14.7%) and its low proportion of fine particles (26.2%).
Olive leaves showed the lowest NDFd (3.0%). The caecal ammonia concentration in this diet was very low (2.4 mmol l^{-1}), below the requirements for maximum growth rates of rumen bacteria, 3.6 mmol l^{-1} according to SATTER and SLYTER (1974), which might limit microbial activity. Furthermore, its high lignin concentration (9.5%) could also partially explain the low value obtained for NDFd. Caecal VFA concentration in this diet was not very different to NaOH-treated straw and sunflower hulls diets, which might indicate a higher fibre digestion than that observed. However, the correlation between caecal VFA concentration and NDFd observed in this study was very low (r=0.20; P=0.25)

Caecal pH

Source of fibre influenced (P<0.001) the excretion of soft faeces (expressed as percentage of body weight, %BW), the DM of caecal contents, the weight of dry caecal contents (%BW), caecal pH and caecal concentrations of VFA and ammonia (Table 2).
A stepwise regression procedure was used to relate caecal pH with chemical and particle size of fibrous sources. The results obtained (n=58) were:

Step 1: $pH=6.20(\pm0.091) - 0.0058(\pm0.0015)FP$
$R^2=0.219 \qquad P<0.001$

Step 2: $pH=6.72(\pm0.11) - 0.067(\pm0.011)UA - 0.0074(\pm0.0012)FP$
$R^2=0.533 \qquad P<0.001$,

where FP was the proportion of particles lower than 0.315 mm and UA the uronic acid content (%) of the feeds (Table 1).
When the caecal traits were used as independent variables, the regressions equations obtained were:

Step 1: $pH=6.29(\pm0.089) - 0.38(\pm0.075)DCC$
$R^2=0.316 \qquad P<0.001$
Step 2: $pH=6.87(\pm0.13) - 0.011(\pm0.0021)VFA - 0.39(\pm0.062)DCC$
$R^2=0.541 \qquad P<0.001$
Step 3: $pH=6.76(\pm0.14) + 0.0072(\pm0.0036)(N\text{-}NH_3) - 0.011(\pm0.0021)VFA - 0.38(\pm0.060)DCC$
$R^2=0.573 \qquad P<0.001$,
where DCC was the dry weight of caecal contents (%BW) and VFA and N-NH_3 the volatile fatty acid and ammonia concentrations of caecal contents (mmol l^{-1}) (Table 2).
The effect of proportion of fine particles (FP:<0.315 mm) together with the dry caecal contents (DCC, %BW) might be accounted for a longer fermentation time. Caecal pH decreases with dietary uronic acid (UA) content, as it is easily digested in the caecum and accounts for a significant proportion of the substrated fermented. Caecal ammonia and VFA concentrations affected directly the caecal pH (r=0.259, P=0.049 and r=-0.455, P<0.001, respectively).
Rabbits fed paprika meal, olive leaves and soya-bean hulls showed similar values of caecal pH (5.69, on average). Paprika meal and olive leaves had a relatively low UA content (5.4% on average), but the highest proportions of fine particles (93.2 and 62.2%, respectively), which agrees with the high weight of dry caecal contents obtained in these diets (1.45%, on average). The very low ammonia concentration in caecum of rabbits fed olive leaves diet (2.4 mmol l^{-1}) also contributed to explain the pH value. The high UA content and the VFA concentration observed

for soya-bean hulls diet (9.1% and 64.4 mmol l^{-1}, respectively) were the main responsible of the low caecal pH observed.

Caecal pH of lucerne hay (5.83) was higher than that observed for paprika meal and soya-bean hulls, probably due to the lower weight of dry caecal contents (0.87 vs 1.40%).

The high value observed for caecal pH for the NaOH-treated straw diet (6.28) could be accounted for the low UA and caecal VFA concentration (1.9% and 38.2 mmol l^{-1}, respectively). This diet showed a relatively low weight of dry caecal contents (0.76%) and the highest value of ammonia concentration (18.0 mmol l^{-1}).

The sunflower hulls diet showed also a high value of pH (6.09). The low weight of dry caecal content (0.84%), that might be related to its larger particle size, and the high ammonia concentration (16.5 mmol l^{-1}) might explain this result.

From this study it could be concluded that NDFd and caecal pH were mainly affected by the physical properties (particle size) of the fibre source. Further inclusion of lignin and uronic acids contents improved significantly the prediction of NDFd and caecal pH, respectively.

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Efecto del tipo de fibra sobre la digestion de la fibra neutro detergente y parametros celales en conejos - Se utilizaron 60 conejos Neozelandés Blanco x Californiano para determinar el coeficiente de digestibilidad de la FND (CDFND) y estudiar los parámetros cecales (excreción de heces blandas, peso del contenido cecal seco respecto al peso vivo (PCCsPV) y pH y concentración cecal de ácidos grasos volátiles (AGV, mmol l^{-1}) y nitrógeno amoniacal ($N\text{-}NH_3$, mmol l^{-1})) de 6 alimentos fibrosos: torta de pimentón, hoja de olivo, heno de alfalfa, cascarilla de soja, cascarilla de girasol y paja de cebada tratada con sosa. Se formularon 6 dietas que incluían estos ingredientes como única fuente de fibra y un concentrado, sin fibra, a base de almidón, proteína, grasa y un corrector vitamínico mineral. La fuente de fibra afectó significativamente (P<0.001) todas las variables medidas. El rango de variación del CDFND fue de 3.0 a 35.1%. Un análisis de regresión paso a paso se realizó para obtener una predicción de este coeficiente (n=60) usando como variables independientes la composición química, el tamaño de partícula (PL: partículas largas, mayores de 1.25 mm; PF partículas finas, menores de 0.315 mm) y los parámetros cecales: CDFND= 32.93(±3.01) + 0.29(±0.15) ($N\text{-}NH_3$) - 1.27(±0.23) Lignina - 1.83(±0.27) PL (R^2=0.627; P<0.001). El pH cecal presentó un rango de variación entre dietas de 5.61 a 6.28. Mediante un análisis de regresión paso a paso se obtuvieron dos ecuaciones (n=58) que explicaban esta variable, utilizando la composición química y tamaño de partícula de la fuente de fibra o los parámetros cecales medidos, respectivamente: pH= 6.72(±0.11) -0.067(±0.011) AU - 0.0074(±0.0012) PF (R^2=0.533; P<0.001), siendo AU el porcentaje de ácidos urónicos, y pH= 6.76(±0.14) + 0.0072 (±0.0036) ($N\text{-}NH_3$) - 0.011(±0.0021) AGV - 0.38(±0.060) PCCsPV (R^2=0.573; P<0.001).
