Proceedings of the

4-7 july **2000** – Valencia Spain

These proceedings were printed as a special issue of WORLD RABBIT SCIENCE, the journal of the World Rabbit Science Association, Volume 8, supplement 1

ISSN reference of this on line version is 2308-1910
(ISSN for all the on-line versions of the proceedings of the successive World Rabbit Congresses)

**BELENGUER A, BALCELLS J., FONDEVILA M.,
TORRE C. , GUADA J. A.**

EFFECT OF DIETARY CARBOHYDRATES ON CAECOTROPHES PRODUCTION. PURINE DERIVATIVES METHODS AGAINST FAECAL COLLECTION

Volume C, pages 101-107

EFFECT OF DIETARY CARBOHYDRATES ON CAECOTROPHES PRODUCTION. PURINE DERIVATIVES METHODS AGAINST FAECAL COLLECTION

BELENGUER[1] A, BALCELLS[1] J., FONDEVILA[1] M., TORRE[2] C. , GUADA[1] J. A.

[1]Departamento de Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria. Miguel Servet 177. 50013 . Zaragoza. Spain. E-mail: balcells@posta.unizar.es
[2]Purina España. S.A. Sant Joan 193; Barcelona.Spain.

ABSTRACT

The present study compares estimates of caecotrophes production either from preventing caecotrophy by using neck collar or from urinary purine derivatives (PD) excretion. A total of 64 New Zealand growing male rabbits were used to study the effect of diet composition on caecotrophes production. Diets were formulated using two sources of non-structural carbohydrates (barley, B and corn, C, grains), included at two proportion: High (40-45 %; HB or HC) or low (12-14 %; LB or LC) and the four combinations were given either with alfalfa hay (AH) or sugar beet pulp (SBP). Diets were given "ad libitum". Growth rate, dry matter intake and digestibility were not modified by the cereal source, although diets with a high proportion of non structural carbohydrates promoted a higher growth rate (26.4 vs. 19.8; $P<0.05$). Between fibre sources, alfalfa hay allowed for a higher intake level and growth rate than sugar beet pulp (118.6 and 25.6 vs 104.2 and 20.4 g/d). N recycling values were higher considering data derived from PD excretion than preventing caecotrophy (1.99 vs 1.02 g/d) although caecotrophes production estimated by both methodologies responded similar to the experimental treatments. Caecotrophes production was higher in animals fed high level of structural carbohydrates (2.64 vs 1.15 g/d) and in alfalfa hay than SBP (2.54 vs 1.33) as a source of SCH.

INTRODUCTION

Rabbits, like other caecotrophyc animals, are single-stomached but the reingestion of a significant amount of the faeces - soft faeces or caecotrophes - enables them to reutilize the microbial protein synthesized in the caecum.Therefore, the rabbit caecum is a fermentation chamber susceptible to be manipulated to optimise protein supply to the host animal.

Various approaches and techniques are available for the study of microbial contribution to total rabbit intake. Avoiding caecotrophy by using a neck collar is the conventional choice and it has been employed extensively. However, quantitative evaluation of caecotrophy is still the largest element of uncertainty in rabbit nutrition. Current measurements are of limited value due to the fact that avoidance caecotrophy is incompatible with normal feeding behaviour in the sense that caecotrophy is a integral part of the rabbit digestive physiology. Recently an indirect methodology based on the urinary excretion of purine derivatives (PD)

has been developed, allowing for estimate microbial protein intake (Balcells et al. 1998). In the present experiment, values from PD excretion are validated contrasting them against values of microbial N obtained by conventional method, when caecal fermentation was modified by changing type and ratio (structural/non structural) of dietary carbohydrates.

MATERIAL AND METHODS

Eight experimental diets were formulated to study the effect of type and level of dietary carbohydrates on caecotrophes production. Two sources of non-structural carbohydrates (NSC), barley (B) and corn grain (C), were used, both included at two levels (LCH), high (40-45 %; HB or HC) or low (12-14 %; LB or LC) and the four combinations were given either with alfalfa hay (AH) or sugar beet pulp (SBP) as major sources of structural carbohydrates (SC) (Table 1). Diets were given "ad libitum". Sixty four New Zealand white male rabbits, with a mean initial live weight of 1.5-1.6 kg, aged 45 d, were randomly allocated within the experimental diets and divided in eight feeding groups. They were penned individually during the adaptation period, with free access to drinking water and maintained in metabolism cages for faeces and urine collection.

Table 1. Composition of experimental diets based on corn (C) and barley (B) grain as non-structural carbohydrates (NSC) and sugar beet pulp {SBP} and alfalfa hay {AH} as structural carbohydrates (SCH) incorporated at two inclusion levels (LCH: low starch {LS: LC or LB} and high starch {HS: HB or HC} diets).

INGREDIENTS (g/100g)	**HBSBP**	**LBSBP**	**HCSBP**	**LCSBP**	**LBAH**	**HBAH**	**LCAH**	**HCAH**
Corn grain			40.98	12.38			13.41	40.53
Barley grain	44.03	14.86			14.85	44.03		
Alfalfa Hay	8.25	17.34	8.43	17.33	51.49	24.02	52.04	24.82
Sugar Beet Pulp	23.96	51.49	25.62	51.5	16.84	8.23	17.35	8.36
NH_3 treated straw	5	4.95	5.12	4.95	4.95	6	4.98	6.08
Soyabean meal	16.91	9.49	18.93	11.96	9.9	15.01	10.04	17.48
Vit-min supl.	1.85	0.89	0.92	0.909	0.98	2.7	1.19	2.729
Sunflower oil		0.98		0.99	0.99		0.99	

Each experimental period lasted for twenty-one days, the first fifteen days for adaptation to the experimental diet. After, rabbits were kept in metabolism crates for four days faeces and urine collection. For each experimental period, the following schedule was adopted: adaptation to the metabolic cage on days 1 and 2, digestion trial on days 3 to 6, and the last twenty-four hours (day 7) rabbits were fitted with a neck collar (50 mm i.d -270 mm o.d and weighing aprox. 67 g) to prevent caecotrophy, and caecotrophes were collected together with hard faeces. Once the trial was finished four animals of each experimental group were slaughtered between 8:00 and 11:00. Animals were weighed once weekly, and also at the beginning and end of the experimental period.

Daily urine was collected over H_2SO_4 (100 ml/l, final pH<3) and weighed, bulked for each animal and stored at –20ºC. Faeces and caecotrophes were collected and frozen immediately at –20ºC. Once the animals were killed by cervical dislocation, the caecum was excised,

weighed and a sample of microbial population was obtained, using methyl-cellulose as a detaching agent (Martín-Orúe et al., 1998). Urinary PD (allantoin, uric acid, hypoxanthine and xanthine) and PB (adenine and guanine) were analysed by reverse-phase HPLC, using two Spherisorb C-18 ODS-2 (4.6x250 mm) columns following Martín-Orúe et al. (1995). Duodenal purine bases (PB) were calculated using Balcells et al. (1998) model, microbial purine bases were calculated by difference between total flow of PB and dietary PB and N-recycling was calculated using PB as a microbial marker and taken PB/N ratio into caecotrophes as a reference.

Results were analysed by ANOVA as a factorial design 2x2x2 being the main effects: type of non-structural (NSC: B *vs*. C) or structural (SCH: AH *vs*. SBP) carbohydrates and level of non-structural carbohydrates (LCH: high *vs*. low). Interactions between different factors were also analysed (NSC effect against LCH; SC against LCH, and NSC against SCH) although only SC against LCH did reach statistical significance and is presented in the tables.

RESULTS AND DISCUSSION

In table 2 are presented growth rate, dry matter intake, digestibility, and N excretion as soft faeces. Mean growth rate was 23.06±0.97 g/d, from and initial weight at 45 days of age of 1.6±0.0 to 2.1±0.2 kg when animals were slaughtered at 66 days. Inclusion of high levels of starch promoted the highest growing rate ($P<0.001$) and the lowest feed conversion rate. Between starch sources, C diets only tended to promote a higher growth rate than those including B (24.5 *vs* 21.6 g/d). Differences between SC were evident, AH diets showing higher growth rates than those formulated with SBP (25.6 *vs* 20.4). Average DM intake was 112 g/d (2.1 to 2.3 times maintenance requirements) and it was higher in AH than in SBP diets (118 vs. 104 g/d; $P<0.05$) being such differences more pronounced at the highest level of SC inclusion. No effect on DM intake was observed related to the type or level of starch inclusion. Differences among AH and SBP diets agree well with existing literature (Carabaño et al. 1997) and it would be explained by some difficulties in gastric evacuation of pulp.

Digestibility of DM is also presented in Table 2. Rabbits eating diets with a high proportion of NSC or using SBP as a source of SC showed a higher DM digestibility than those with low proportion of NSC or AH diets (71.72 and 71.6 *vs*. 65.3 and 65.5 %; es= 1.87; $P<0.001$). However, no differences were detected between C and B as sources of NSC. Indeed, changes in digestibility reflect differences in starch/fibre composition among diets and SBP is a more digestible substrate than AH because of its low content of ADF, lignin and also for a more digestible NDF fraction (Giddene et al., 1987).

Despite of the size of the collar, in some cases animals were still able to eat soft faeces, which was detected from the absence of caecotrophes in the collector. Fitting neck collar had a significant effect on rabbits nutritive behaviour and dry matter intake, decreasing significant and constantly (between 15 to 57 % of total dry matter intake) with a strong individual

variation in such parameter. Furthermore, not always it was easy to discriminate soft from hard faeces because some kind of transition was observed among them, so data in relation to soft faeces need to be considered with caution. Average DM excretion as caecotrophes was 18.01±0.81g, and the value was in the range described in literature when data for 24 h collection are taken into account (Carabaño et al. 1988). Crude protein concentration in caecotrophes was much higher than in hard faeces (31.7±0.43 vs 15.8±0.26 %) whereas the opposite was observed for NDF (37.1± 0.47 vs 65.5±0.32 %). Crude protein content in the caecotrophes was independent of the experimental diet although N excretion with soft faeces was affected by the experimental treatment. Caecotrophes production increased with structural carbohydrates and this was particularly evident when alfalfa was the fibre source.

Table 2. Effect of dietary inclusion of barley or corn (B and C) and alfalfa hay or sugar beet pulp (AH and SBP) as sources of non-structural (NSC)[A] and structural carbohydrates (SCH)[B], respectively, given at two levels of inclusion of NSC (LCH: HB and HC or LB and LC, respectively) on growth rate, dry matter intake and digestibility, N in caecotrophes and recyclage throughout the cecotropy process in growing rabbits.

		Type of NSC		**Type of SCH**			**Signification effect**	
	CHS level	B	C	AH	SBP	SD	SCH	LCH
Growth rate (g/d)	High NSC	26.1	26.7	28.2	24.5	7.68	**	***
	Low NSC	17.3	22.4	23.1	16.6			
	$\bar{x}$	[21.6]	[24.5]	[25.6]	[20.4]			
DM intake (g/d)	High NSC	116.1	107.8	117.3^{b}	106^{b}	18.99	***	NS
	Low NSC	112.3	112.9	127.7^{a}	97.5^{c}			
	$\bar{x}$	[114.1]	[110.4]	[122.5]	[101.6]			
DM digestibility	High NSC	71.4	72	70^{a}	73.5^{a}	5.31	***	***
	Low NSC	65.4	65.3	61^{c}	69.8^{b}			
	$\bar{x}$	[68.3]	[68.7]	[65.5]	[71.6]			
N excretion in Caecotrophes(g/d)	High NSC	0.83	0.88	1.01	0.65	0.323	***	*
	Low NSC	1.08	0.91	1.11	0.86			
	$\bar{x}$	[0.95]	[0.90]	[1.06]	[0.77]			

A: the effect of non-structural carbohydrates never reaches statistical significance and was no included in the table; B: when the interaction (LCHxSCH) effect did reach statistical significance values appear with different subscripts (a.b.c). SD: standard deviation; SCH, SCH, LCH: statistical significance of type of NSC, type of SCH and NSC level effects, respectively. NS, no significant; ∗, $P < 0.05$; ∗∗, $P < 0.01$; ∗∗∗, $P < 0.001$.

The urinary excretion of PD, the duodenal flow of total and microbial PB and the N-recycling derived from PD method are presented in Table 3. Allantoin constitutes the main urinary PD (91 % of total excretion) followed by uric acid (9 %) whereas xanthine and hypoxanthine only appear in trace amounts. Urinary excretion of PD averaged 1,247±60 µmol/Kg $PV^{0.75}$, which was much higher than the endogenous losses reported previously (588±40 µmol/Kg $PV^{0.75}$; Balcells et al., 1998), considered as the threshold level required to maintain a constant urinary recovery of the duodenal PB. Thus, it seems acceptable that the response in urinary PD could be attributable to the exogenous input of duodenal PB.

Urinary excretion of PD derivatives was significantly modified by the experimental treatment, mainly explained by allantoin excretion. Daily excretion of PD and allantoin was higher in animals fed on diets with a high proportion of SC (allantoin and total PD, 1,683 and

1,886 *vs* 2,164 and 2,518 µmol/d; P<0.05 for diets high NSC *vs* low NSC respectively). Average excretion of uric acid, independent of the experimental treatment, was 188.7±9.4 µmol/d. Duodenal flow of purine bases closely reflects changes in urinary PD excretion although when duodenal flow was corrected by dietary PB input differences among treatments were still evident. Intake of microbial PB were higher in animals fed on the highest proportion of SC (1,505 *vs* 667 µmol/d; P<0.01) and between these diets those given AH showed a higher level of microbial PB intake than those given SBP, however this effect was not constant and animals fed on the low level of SC showed the oposite trend.

Table 3. Effects on urinary excretion of PD, duodenal flow of purine bases and N recycling by caecotrophy process.

		Type of NSC		Type of SCH			Signification effect	
	CHS level	B	C	AH	SBP	SD	SCH	LCH
PD	High NSC	1,999.9	1,774.3	$1,891.7^{c}$	$1,881.8^{c}$	868.52	**	*
(µmol/d)	Low NSC	2,167.5	2,556.6	$2,986.2^{a}$	$2,362.1^{b}$			
	$\bar{x}$	[2,086.4]	[2,178]	[2,439]	[1805]			
Duodenal PB	High NSC	1.556	1.175	1.329^{b}	1.408^{b}	1.2403	**	**
	Low NSC	2.063	2.308	3.131^{a}	1.256^{c}			
	$\bar{x}$	[1.810]	[1.780]	[2.230]	[1.327]			
Microbial PB	High NSC	0.815	0.518	0.532^{c}	0.822^{b}	1.146	*	**
Intake	Low NSC	1.362	1.629	2.218^{a}	0.792^{b}			
	$\bar{x}$	[1.089]	[1.111]	[1.375]	[0.806]			
N recycling	High NSC	1.386	0.882	0.998^{c}	1.336^{b}	2.2563	*	*
[urinary PD]	Low NSC	2.282	2.999	3.986^{a}	1.331^{b}			
	$\bar{x}$	[1.815]	[2.136]	[2.547]	[1.333]			

A: the effect of non-structural carbohydrates never reaches statistical significance and was not included in the table; B: when the interaction (LCHxSCH) effect did reach statistical significance values appear with different subscripts (a,b,c). SD, standard deviation ; NSC, SCH, LCH, statistic significancy of type of NSC, type of SCH and NSC level effects, respectively; NS, no significant; ∗, (P < 0.05); ∗∗, (P < 0.01); ∗∗∗, (P < 0.001).

Figure 1. N recycling values derived from preventing caecotrophy using a neck collar ▭ or from urinary PD excretion ▭ , and dietary N intake ▭ in rabbits receiving diets formulated with barley or corn (B and C) and alfalfa hay or sugar beet pulp (AH and SBP) as sources of non-structural (NSC) and structural carbohydrates (SCH), respectively, given at two levels of inclusion (LCH: High NSC and Low NSC).

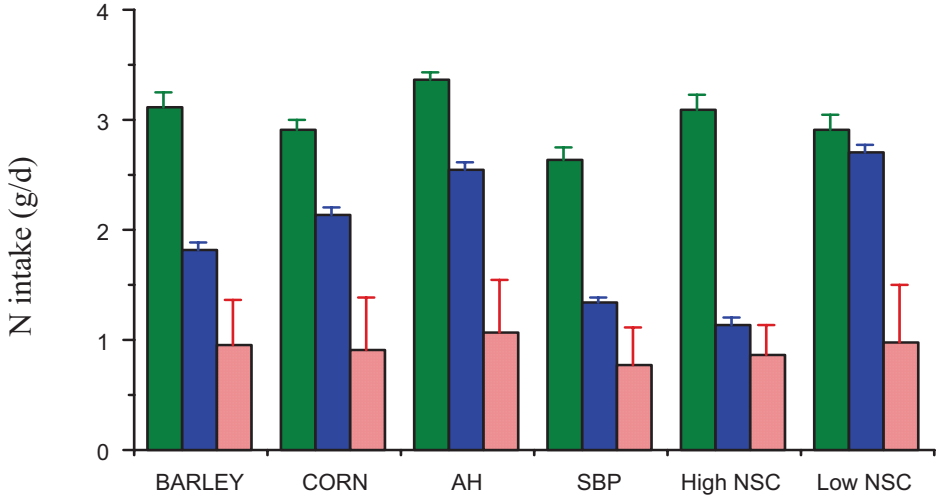

Figure 1 shows the N recycling by the caecotrophy process using both systems: a) caecotrophes collection using neck collar to prevent caecotrophy and b) values derived from urinary excretion of PD. In the latter, N recycling is calculated by using duodenal flow of microbial PB and PB/N ratio in the caecotrophes. PB/N ration in the soft faeces averaged 0.6±0.054 mmol/g and composition was constant. In general, caecotrophes consumption estimated by the PD method gave higher values that caecotrophy prevention (1.99 vs 1.02 g/d) although changes induced by the experimental treatment showed the same pattern using both methodologies. In any case, both systems confirmed the significancy of the caecotrophy process in rabbit protein nutrition, although it is difficult to conclude what is the true value of N recycling or at least which is the best method, given that a reference standard does not exist.

AKNOWLEDGEMENTS

Mr. A. Belenguer has been granted by the Institución Fernando el Católico (Diputación Provincial de Zaragoza). This work was financed by Purina España S.A. (Pr. OTRI 96/0040).

REFERENCES

BALCELLS J., GANUZA J.M., PÉREZ J.F., MARTÍN-ORÚE S.M. AND GONZALEZ RONQUILLO M. (1998) Urinary excretion of purine derivatives as an index of microbial-nitrogen intake in growing rabbits. *British Journal of Nutrition* **79**, 373-380.

BELLIER R., GIDENNE T., VERNAY M. AND COLIN M. (1995). In vivo study of circadian variations of the cecal fermentation pattern in postweaned and adult rabbits. *Journal of Animal Science* **73**, 128-135.

CARABAÑO R., FRAGA M.J., SANTOMA G. AND DE BLAS J.C. (1988). Effect of diet on composition of cecal contents and on excretion and composition of soft and hard feces of rabbits. *Journal of Animal Science* **66**, 901-910.

CARABAÑO R., MOTTA-FERREIRA W., DE BLAS J.C. AND FRAGA M.J. (1997). Substitution of sugarbeet pulp for alfalfa hay in diets for growing rabbits. *Animal Feed Science and Technology* **65**, 249-256.

GIDENNE T. (1987). Effet de l'adition d'un concentré riche en fibres dans une ration à base de foin, distribuée à deux niveaux alimentaires chez la lapin adulte. 2. Mesures de digestibilité. *Reproduction, Nutrition and Development* **27**, 801-810.

MARTÍN ORÚE S.M., BALCELLS J., GUADA J.A. AND CASTRILLO C. (1995). Endogenous purine and pyrimidine derivative excretion in pregnant sows. *British Journal of Nutrition* **73**, 375-385.

MARTÍN ORÚE S.M., BALCELLS J., ZAKRAOUI F. AND CASTRILLO C. (1998). Quantification and chemical composition of mixed bacteria harvested from solid fractions of rumen digesta: effect of detachment procedure. *Animal Feed Science and Technology* **71**, 269-282.