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EFFECT OF THE SOURCE AND LEVEL OF CEREAL IN DIET ON THE RABBIT CAECAL ENVIRONMENT AND MICROBIAL POPULATION

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ABSTRACT

The effect of the cereal type and dietary level was studied on fibre digestibility, caecal environmental and population characteristics using 32 rabbits, randomly allocated to 4 diets based on barley (B) or corn (C) as cereal sources, included at two levels (high, H, or low, L), and with alfalfa hay as the main fibre source. After a digestibility trial (8 rabbits per diet), 4 animals of each group were slaughtered (between 9:30 and 11:00), and caecal environmental parameters were recorded. There was no effect of the type of cereal on NDF digestibility, but it was higher in L diets (355 vs. 298 g/kg; $P<0.001$). Caecal content weight was also larger in L ($P <0.01$). Diet did not affect caecal pH and ammonia or volatile fatty acid concentrations. However, proportion of acetate increased, and butyrate decreased, in L compared with H. Total bacterial numbers ($x10^7$/ml) were higher in B than in C (162 vs. 40; $P<0.05$), differences being greater at H level. Cellulolytic counts ($x10^5$/ml) were inversely related to the level of B (20 vs. 3 for LB and HB; $P<0.01$), but the opposite was observed in C. The cereal level in diet affects bacterial population, decreasing NDF digestibility and changing volatile fatty acid proportions.

INTRODUCTION

The anaerobic symbiotic flora from the caecum enables the rabbit to take advantage of fibrous feeds, at the time it serves as an additional protein input by caecotrophy. Because of caecal location in the digestive tract, mainly fibrous particles and endogenous material are found in caecum contents, with a scarce contribution of starch and other digestible dietary components. Microbial fermentation in the caecum is lower than in ruminants (Jehl et al., 1995) or other hindgut fermentors, like horses. Rate of passage and the specific selection mechanism that avoids the entrance of large fibrous particles in the caecum may be implicated in this response (deBlas, 1984), in addition to intrinsic differences in the flora. In any case, the volatile fatty acids (VFA) produced by microbial caecal fermentation may contribute from 12 to 40 % to the overall energy requirements (Hoover and Heitman, 1972; Marty and Vernay, 1984).

The intensive systems applied in rabbit production, using high concentrate diets, restrict the potential of caecal fermentation, depending on the source of cereal and its level of inclusion in diet, and have been associated to higher risks of enteritis (Bellier and Gidenne, 1996). A higher arrival of starch to the caecum may affect environmental conditions and cellulolytic populations, and therefore alter fibre digestion. Boulahrouf et al. (1991) observed a lower cellulolytic population when the level of dietary fibre was reduced.

In this work, the effect of the source and level of cereal included in diet over the caecal environment, microbial population and fibre fermentation is studied.

MATERIAL AND METHODS

Thirty-two New Zealand male rabbits, of about 45 days and weighing between 1.5 and 1.6 kg, were allocated to four diets, based on a 3:1 ratio of alfalfa hay and sugar beet pulp as fibrous feeds, and either barley (B) or corn (C), at two levels of inclusion (high, H, and low, L), resulting in neutral detergent fibre (NDF) proportions of 28-29 and 40-41 %, respectively (TABLE 1). Animals were allocated in individual cages and received the experimental diets “ad libitum” for 14 days. Then, they were adapted for two days to metabolic cages before the start of the balance of digestibility, that lasted 4 days. After the balance trial, 16 animals (4 per diet) were placed in individual cages for another 5 days receiving the same previous diet, before being slaughtered by cervical dislocation.

TABLE 1: Ingedients (%) and chemical composition (g/kg dry matter) of experimental diets

	diet LB	**diet HB**	**diet LC**	**diet HC**
Ingredients:				
Alfalfa hay	51.5	24.0	52.0	24.8
Sugarbeet pulp	16.8	8.2	17.4	8.4
Barley	14.9	44.0	------	------
Corn	------	------	13.4	40.5
Treated straw	5.0	6.0	5.0	6.1
Soya meal	9.9	15.0	10.0	17.5
Others	2.0	2.7	2.2	2.7
Chemical composition:				
Organic matter	911	921	912	931
Crude protein	173	178	168	166
Ether extract	27	18	31	27
Neutral detergent fibre	403	293	409	275
Starch	109	239	117	341

After the slaughter (between 9:30 and 11:00; 4 rabbits per day), the digestive tract was removed, and each section tied and excised. Volume of caecum was determined by water displacement, and full and empty weights of the organ were measured. After recording pH, caecal contents were sampled for different determinations: bacterial (10 g content) and protozoal concentrations (2.5 g in 2.5 ml 18% formalin), ammonia (1 g in 1 ml 0.2N HCl) and volatile fatty acid (VFA; 1 g in 1 ml 0.5M H_3PO_4 plus 50 mM 3-methyl valeric acid).

Chemical analysis of diets, and feed refusals and faeces from the balance trial were determined according to the AOAC (1980) and Van Soest et al. (1991) procedures. Ammonia was determined according to the colorimetric method of Chaney and Marbach (1962), and volatile fatty acids by GLC according to Jouany (1982). Sample for bacterial counts was processed aseptically under a continuous flow of CO_2, being diluted immediately in anaerobic dilution solution (Bryant and Burkey, 1953) and then serially diluted. One ml of dilutions 10^{-5} to 10^{-10} were inoculated in triplicated tubes containing total culture media with ball-milled cellulose for simultaneous determination of total (by pH) and cellulolytic (by visual disappearance of cellulose) bacterial counts by the MPN procedure (Dehority et al., 1989), after incubation for 14 days at 39 ºC. Also, dilutions 10^{-2} to 10^{-4} were used to inoculate duplicated MacConkey agar plates, incubated for 24 h at 38 ºC for coliforms enumeration.

Results were subjected to analysis of variance according to a factorial design 2 x 2, with 8 (digestibility) or 4 (the other parameters) data per experimental treatment.

RESULTS AND DISCUSSION

Rabbit live weight at slaughter, volume and content weight of caecum and NDF digestibility of diets are shown in TABLE 2. Rabbit weight did not differ between diets. The NDF digestibility decreased with the level of cereal inclusion ($P < 0.001$). Although the alfalfa:sugar beet pulp ratio was kept constant for all diets (3:1), the selective activity of caecum against large fibrous particles might increase the proportion of beet pulp into the organ in L, thus affecting NDF digestibility. This can also explain a physical adaptation to high dietary fibre by increasing caecal volume ($P < 0.10$) and content weight ($P < 0.01$), although bibliography in this regard is contradictory. None of these parameters was affected by the source of cereal.

Environmental caecal characteristics are summarised in TABLE 3. Neither pH values nor ammonia concentrations were affected by diet composition ($P > 0.05$). However, Morisse et al. (1985) and Gidenne (1995) observed an increase in pH and caecal ammonia when dietary NDF increased from 29 to 37% and from 23 to 32%, respectively, being the slaughter time in the latter close to that in our experiment. Total VFA concentration was also unaffected by diet characteristics ($P > 0.05$), but proportion of acetate was higher, and that of butyrate lower, in L than in H diets, in agreement with Gidenne (1995). Differences between levels in acetate proportions were only observed in corn diets. Corn diets also tended to give higher isovalerate proportions than barley (average values 0.35 and 0.13 %, respectively; P <0.10).

TABLE 2: Weight at slaughter (kg), caecum volume (ml) and content weight (g) and NDF digestibility (g/kg) of rabbits fed on diets with barley or corn at two different levels.

	diet LB	**diet HB**	**diet LC**	**diet HC**	**r.s.d.**
slaughter weight	2.42	2.51	2.52	2.57	0.14
caecal volume [a]	129	98	144	110	32
caecal content [b]	107	74	111	82	20
NDF digestibility [c]	357	299	354	296	44

r.s.d.: residual standard deviation

a) level effect: P <0.10; b) level effect: P <0.01; c) level effect: P <0.001

TABLE 3: Caecal pH, ammonia and VFA concentration (mmol/L) and proportions of the main VFA (%) in rabbits fed on diets with barley or corn at two different levels of inclusion.

	diet LB	**diet HB**	**diet LC**	**diet HC**	**r.s.d.**
pH	6.15	6.17	6.01	6.14	0.24
ammonia	3.60	2.74	3.65	3.20	0.18
VFA	65.6	64.0	68.5	60.5	9.8
- acetate [a b]	80.1	77.7	80.3	73.2	2.4
- propionate	6.3	6.1	6.9	8.1	1.6
- butyrate [a]	12.5	15.1	11.4	16.6	2.0

r.s.d.: residual standard deviation.

a) level effect: P <0.01; b) interaction: P <0.10.

As expected, no protozoa were found in the caecum of any of the animals studied. Noticeable concentrations (between 10^2-10^3/g) of coliforms were only observed in two rabbits given diet HB, in opposition with Morisse et al. (1985), who give average concentrations between 10^2 and 10^4/ml. Total and cellulolytic anaerobic bacteria, expressed as concentration or as absolute numbers in the caecal content, are summarised in TABLE 4. A significantly higher (P <0.05) concentration of total bacteria was observed in barley diets compared with corn diets. The level of inclusion of B tended to increased total concentration (P <0.10), whereas no effect was observed in C diets. Cellulolytic concentration was inversely related to the level of barley (P <0.01), as reported by Boulahrouf et al. (1991), but no significant differences were observed between levels of C. This trends in cellulolytic bacteria increased numerically when population was expressed as total numbers in the organ, but the high magnitude of the error term (variation coefficient of 82 %) avoids the appearance of significant differences. Available analysis of caecotrophes from this experiment (data not shown) indicates no changes in NDF concentration among diets, which, assuming the direct relationship between caecotrophes and caecum composition (Carabaño et al., 1988), differences in microbial populations cannot be attributable to different caecal NDF content.

A high inclusion of barley promotes an increase in total bacterial concentration in the caecum, but decreases cellulolytics, leading to a shift in the type of fermentation to a less acetate-more butyrate proportions, even at similar VFA concentrations. Among the mechanisms involved in the higher NDF digestibility observed with L diets are an increase in the caecum size and in the bacterial cellulolytic counts. However, in corn diets, there were no significant response in bacterial population to the dietary level of cereal, as in B, eventhough the changes in VFA proportions and the decrease in NDF digestibility when increasing the level of C are also noticeable.

TABLE 4: Total and cellulolytic counts of caecal anaerobic bacteria in rabbits fed on diets with barley or corn at two different levels of inclusion.

	diet LB	diet HB	diet LC	diet HC	r.s.d.
Concentration:					
- Total (x 10^7/g) [a b]	85.2	238.8	75.8	3.3	54.0
- Cellulolytic (x10^5/g) [c]	19.7	3.1	6.7	15.1	4.2
Absolute numbers:					
- Total (x 10^{10}) [d b]	8.62	17.04	8.07	0.29	4.41
- Cellulolytic (x 10^8) [e]	2.16	0.23	0.89	1.40	0.48

r.s.d.: residual standard deviation.

a) cereal effect: P <0.05; b) interaction: P <0.10; c) interaction: P <0.01; d) cereal effect: P <0.10; e) interaction: P <0.05.

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