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EFFECT OF DIETARY SODIUM ON DIGESTIBILITY OF NUTRIENTS AND PERFORMANCE IN GROWING RABBITS

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Abstract: The effects of a reduction in the sodium content from 2.6 to 1.6 g/kg DM in diets with similar electrolyte balance on fattening performances and ileal apparent digestibility of nutrients were investigated in early weaned rabbits. Two diets containing 17.9% DM of crude protein, with the same composition and only differing in sodium chloride content (0.5 and 0.2%) were given to rabbits weaned at 25 days to measure fattening performance (46 rabbits per diet), and apparent ileal digestibility (AID, 14 rabbits per diet) of DM, crude protein (CP) and amino acids (AA). Neither AID of DM nor AID of CP, which amounted 47.4 and 60.9% as average respectively, were affected by the dietary sodium content. The effect of a decrease in dietary sodium concentration on the AID differed among amino acids, as a reduction was observed for cystine (by 11%; $P<0.01$), methionine, arginine, tyrosine and phenylalanine (by 4.9, 5.6, 7.4 and 10.3%; respectively $P<0.05$), glutamic acid, leucine and isoleucine (by 4.1, 6.1 and 7.1%, respectively $P<0.1$), whereas no differences ($P>0.10$) were detected for the other amino acids. A reduction of dietary sodium content did not affect daily weight gain, feed intake and feed efficiency (46.7 g/d, 76.7 g/d and 0.609 as average, respectively) neither during the first two weeks after weaning when the experimental diets were used, nor when the whole fattening period was regarded. The results of this experiment indicate that is possible to reduce the current dietary sodium recommendations for fattening rabbits to 1.6 g/kg DM during the first two weeks after weaning, without impairing either the fattening performances or the ileal digestibility of DM and CP. In addition, this study indicate that the level of sodium in the diet affects the gut absorption of methionine and cystine and several non-essential AA.

Key words: Dietary sodium, amino acid, ileal digestibility, growing rabbits

INTRODUCTION

It is worldwide accepted that sodium is involved in the regulation of pH and osmotic pressure in the cells. Sodium content of the diet is also essential for the absorption of luminal nutrients as glucose and amino acids (Schultz and Zalusky, 1964 and 1965), which are transported across the brush border membrane of the intestinal epithelial cell by several systems that require sodium as a cotransport.

The practical recommendations of dietary sodium vary between 2.0 and 3.0 g/kg DM (Mateos and De Blas, 1998). However, the experimental evidence (Surdeau *et al*., 1976; Harris *et al*., 1984) to establish the recommendation of this mineral is weak and based on growth responses, and no information is available concerning its effect on the digestibility of nutrients in rabbits. Sodium requirements might change when some amino acids become limiting as occurs in pigs (Austic and Calvert, 1981; Patience *et al*., 1987), and particularly when low protein diets are used.

The aim of the present investigation was to determine the effect of a reduction in the sodium content from 2.6 to 1.6 g/kg DM in diets exceeding present recommendations in chloride and other essential minerals, on fattening performances and ileal apparent digestibility of nutrients.

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MATERIALS AND METHODS

Diets

A basal diet (LNA) with low sodium and sufficient chloride content (1.6 and 7.3 g/kg DM, respectively) was formulated according to De Blas and Mateos (1998) and recent works on optimal starter feed composition (Gutiérrez *et al.,* 2002a and b; 2003). A similar diet with high sodium content (HNA) was made by adding to the basal diet 0.3% of sodium chloride resulting in a sodium and chloride content of 2.6 and 9.24 g/kg DM, respectively. The diets were pelleted and included 5 g/kg of lucerne hay marked with an indigestible marker (ytterbium) according to the procedure described by García *et al.* (1999). The ingredients and chemical composition of the basal diet is shown in Table 1.

Ileal digestibility trial

Twenty eight rabbits weighing 473 ± 17 g at 25 days of age were blocked by litter and assigned at random to the experimental diets (14 rabbits per diet) to determine the apparent ileal digestibility of DM, CP and amino acids. Following a 10-days adaptation period, animals were slaughtered by cervical dislocation. Slaughter time was between 19:00 and 21:00 h to avoid the influence of caecotrophy on the chemical composition of the digesta (Merino and Carabaño, 2003). The last 20 cm of the ileum were taken and the ileal contents were removed, frozen and freeze-dried. The samples were then ground and, because of the small quantity available, they were pooled in groups of two rabbits of the same treatment to analyse CP and ytterbium. To determine the amino acid content of the ileal digesta, a fixed amount (0.05 g) of all the samples belonging to each treatment were pooled. Ytterbium content of experimental diets and ileal digesta were analysed to calculate apparent ileal digestibility of CP and amino acid (CP_{id} and AA_{id}) according to the following equation:

CP_{id} = [1 – (dietary ytterbium concentration × ileal CP concentration/ileal ytterbium concentration × dietary CP concentration)] × 100.

AA_{id} = [1 – (dietary ytterbium concentration × ileal amino acid concentration/ileal ytterbium concentration × dietary amino acid concentration)] × 100.

Fattening trial

Ninety two New Zealand White × California mixed-sex rabbits (46 per diet), weaned at 25 days of age and weighing 491 ± 11 g, were blocked by litter and assigned at random to the treatments. After weaning, rabbits were individually caged and were fed the experimental diets throughout a two-week period. After 39 days of age, all the animals received a commercial feed (CUNIUNIC®, NANTA, S.A.: 170 g CP, 5 g sodium chloride, 144 g starch, 373 g NDF and 49 g ADL per kg) until they reached 56 days of age. Animals had *ad libitum* access to the feed and water throughout the whole experimental period. Feed intake, weight gain and mortality rate at day 14 after weaning and at the end of the experimental period were recorded.

Housing

Animals were housed in wire metabolism cages measuring 250 × 600 × 330 mm. A cycle of 12 h of light and 12 h of dark was used throughout the experiment. The light was switched on at 7:30 h. Heating and forced ventilation systems allowed the building temperature to be maintained between 18 and 23ºC throughout the experiment. Rabbits were handled according to the principles for the care of animals in experimentation published by the Spanish Royal Decree 1201/2005 (2005), and following the recommendations for applied nutrition experiments in rabbits of the European Group on Rabbit Nutrition (Fernández-Carmona *et al*., 2005).

Table 1: Ingredients and chemical composition of the basal diet (LNA)

Ingredients (g/kg)		Chemical composition (g/kg DM)[2]	
Wheat	300	Dry matter	907
Wheat bran	140	Ash	77.8
Sunflower meal	50	Crude protein	179
Lucerne hay	370	Starch	227
Lucerne hay-Yb	5	Neutral-detergent fibre (NDF)	335
Wheat straw	89	Acid-detergent fibre (ADF)	173
Lard	30	Acid-detergent lignin (ADL)	46.9
L-Lysine HCl 78	5.7	Gross energy (MJ/kg DM)	18.8
DL-Methionine 99	1.7	Digestible energy (MJ/kg DM)[3]	13.7
L-Threonine	1.6	Sodium	1.60
Sodium chloride	2	Chloride	7.32
Vitamin/mineral premix[1]	5	Potassium	13.1
		Calcium	9.51
		Magnesium	2.25
		Phosphorus	4.2

[1]Provided by Trouw Nutrition España S.A. (Madrid, Spain): Mineral and vitamin composition (mg/kg of feed): Mg, 290; Na, 329; S, 275; Co, 0.7; Cu, 10; Fe, 76; Mn, 20; Zn, 59.2; I, 1.25; choline, 250; riboflavin, 2; niacin, 20; pyridoxine, 1; phytylmetaquinone, 1; alpha-tocopherol, 13; thiamine, 1; retinol, 2.5, and cholecalciferol, 0.019. [2]Amino acid content analysed (g/kg DM): arginine, 8.75; cystine, 3.91; histidine, 3.45; isoleucine, 5.79; leucine, 10.7; lysine, 10.6; methionine, 4.95; phenylalanine, 6.87; threonine 7.59; valine, 7.11; alanine, 7.53; aspartic acid, 15.2; glutamic acid, 29.5; glycine, 7.85; proline, 12.1; serine, 5.99; tyrosine, 4.66. [3]Estimated according to FEDNA (2003).

Analytical methods

Chemical analyses of diets and ileal digesta were performed using the procedures of AOAC (2000) for DM (930.15), ash (923.03), Dumas N (968.06) and starch (according to the alpha-amyloglucosidase method, 996.11). NDF, ADF and ADL were determined according to the sequential method of Van Soest *et al.* (1991). Gross energy (GE) was measured by adiabatic calorimetry. The dietary concentrations of minerals (Na, Cl, K, Ca and Mg) were determined by ion chromatography. Samples were reduced to ashes, dissolved in acid solution and injected into an HPLC system equipped with a conductivity detector and a cation (spherical silica with malic-polybutadiene groups) or anion (polymetacrylate with quaternary ammonium groups) column. Amino acids were determined following acid hydrolysis using a Beckman System 6300HPA amino acid analyzer (Fullerton, CA, USA). Samples were hydrolyzed by reflux in 25 ml of 6 mol/l HCl with 10 g/l added phenol for 24 h at 120°C. For the determination of sulphur amino acids (methionine and cystine), samples were oxidized with performic acid at 0°C for 16 h and then neutralized with 0.5 g of sodium meta-bisulphite before analysis. Tryptophan, being destroyed during acid hydrolysis, was not determined. Ytterbium content of diets and ileal digesta were analyzed by atomic absorption spectrometry (Smith Hieftje 22, Thermo Jarrel Ash, MA, USA) using predosed samples to prepare common matrix standards. Previously, samples were ashed (600°C) and then digested by boiling with a solution of 1.5 mol/l HNO_3 and KCl (3.81 g/l).

Statistical analysis

Data were analyzed as a completely randomized block design with type of diet as main effect and litter as block effect, by using the GLM procedure of SAS (1991). Weaning weight was used as a linear covariate when analysing growth performance.

RESULTS AND DISCUSSION

In our study the dietary electrolyte balance, calculated as Na+K−Cl meq/kg DM, was similar in both diets (189 and 199 meq for diets HNA and LNA). Furthermore, dietary content of chloride in diet LNA was 7.32 g/kg DM which clearly exceeds present recommendations for fattening rabbits (3.11 g/kg DM; De Blas and Mateos, 1998). Accordingly all the dietary effects should be related to differences in Na content.

The effect of treatment on the apparent ileal digestibility (AID) of experimental diets is shown in Table 2. Neither AID of DM nor AID of crude protein (CP), which amounted 47.4 and 60.9% as average respectively, were affected by the dietary sodium content. The effect of a decrease in dietary sodium concentration (from 2.6 to 1.6 g/kg DM) on the amino acids AID differed depending on the amino acids: a reduction was observed for cystine (by 11%; $P < 0.01$), methionine, arginine, tyrosine and phenylalanine (by 4.9, 5.6, 7.4 and 10.3%, respectively; $P < 0.05$), glutamic acid, leucine and isoleucine (by 4.1, 6.1 and 7.1%, respectively; $P < 0.1$), whereas no differences ($P > 0.10$) were detected for other amino acids. These results suggest that a deficit of dietary sodium could impair the efficiency of digestive process and/or the absorption of these amino acids. Early *in vitro* studies in rabbit ileum (Schultz *et al*., 1966, Frizzeli *et al*., 1973) demonstrated that intestinal absorption of amino acids requires the presence of Na in the mucosal solution, which is used as a co-transport. In rabbits, *in vivo* studies relating dietary sodium content and ileal digestibility of nutrients are not

Table 2: The effect of dietary treatments on the apparent ileal digestibility of experimental diets.

	Diets[1]		RMSE[2]	*P*-value
	HNA	LNA		
No. of rabbits	14	14		
Dry matter	47.4	47.5	5.2	0.967
Crude protein	61.2	60.6	5.8	0.871
Arginine	79.9	75.4	3.1	0.018
Cystine	70.7	62.9	4.5	0.007
Histidine	69.9	66.9	4.5	0.254
Isoleucine	71.7	66.6	4.3	0.05
Leucine	73.9	69.4	3.9	0.06
Lysine	77.6	75.4	3.4	0.237
Methionine	78.8	74.9	3.2	0.045
Phenylalanine	66.0	59.2	5.2	0.032
Threonine	67.6	64.0	4.9	0.193
Valine	63.7	68.3	4.8	0.110
Alanine	68.5	64.5	4.7	0.148
Aspartic acid	72.2	68.3	4.2	0.10
Glutamic acid	80.5	77.2	2.9	0.06
Glycine	42.9	46.6	8.4	0.426
Proline	79.3	77.3	3.1	0.271
Serine	61.4	57.2	5.8	0.208
Tyrosine	74.0	68.5	3.9	0.025

[1]HNA = High sodium content (2.6 g/kg DM); LNA = Low sodium content (1.6 g/kg DM). [2]RMSE= Root means square error, number of replicates = 7 (ileal content samples pooled 2 by 2)

available. Studies on pigs (Patience *et al*., 1986; Haydon and West, 1990) observed a relationship between electrolyte balance and apparent ileal digestibility of amino acids, although the mechanisms explaining this effect remain unclear.

The effect of the sodium concentration on growth performance is shown in Table 3. A decrease of dietary sodium content from 2.6 to 1.6 g/kg DM did not affect daily weight gain, feed intake and feed efficiency (46.7 g/d, 76.7 g/d and 0.609 as average, respectively) during the first two weeks after weaning when the experimental diets were used. Dietary treatment neither affected growth traits in the whole fattening period (from 25 to 56 days of age), after a unique commercial diet were fed from 39 to 56 days. No mortality was observed throughout the experiment.

On the overall, a decrease of dietary sodium content reduced the AID of several essential AA but did not affect the growth traits. Consequently the supply of AA of the experimental diets could exceed the requirements for maximal growth. Dietary levels of the most limiting AA (lysine, methionine+cystine and threonine) were higher (by 22, 32 and 7% , respectively) than recommended levels for the overall fattening period (De Blas and Mateos, 1998), in order to assure a correct growth in the starter phase (from 25 to 39 days of age). In the post-weaning period, an excess of nitrogen flow in the ileum has been proven to increase intestinal disorders in early weaned rabbits (Gutiérrez *et al*., 2003; Chamorro *et al*., 2007). The trend in practical formulation in rabbits is to reduce the level of crude protein increasing in parallel that of limiting synthetic AA (lysine, methionine and treonine) to maintain growth responses. In these circumstances, a decrease in the dietary sodium content might result in an impairment of growth performance. The relation between minerals and amino acid requirements has been widely studied in poultry (Austic and Calvert, 1981; Austic and Patience, 1988) and swine (Madubuike, 1980). The inclusion of sodium bicarbonate improved the growth of swine fed lysine-deficient diets (Patience *et al*., 1987). In rabbits, there is not available information concerning the relationship between minerals and amino acids, but our study indicates that dietary sodium content might affect the intestinal absorption of AA.

Table 3: The effect of dietary treatments on growing performance

		Diets[1]		RMSE[2]	*P*-value
		HNA	LNA		
Nº of rabbits		46	46		
Live weight at 25 d (g)		493	488		
Live weight at 39 d (g)		1155	1144	113	0.805
Live weight at 56 d (g)		2027	2012	156	0.829
1st period (25-39 d):	Weight gain (g/d)	47.1	46.2	7.64	0.607
	Feed intake (g/d)	77.1	76.4	12.0	0.848
	Feed efficiency (g gain/g intake)	0.612	0.607	0.061	0.649
2nd period (39-56 d):	Weight gain (g/d)	50.7	52.2	5.8	0.208
	Feed intake (g/d)	163.7	164.8	18.4	0.677
	Feed efficiency (g gain/g intake)	0.312	0.318	0.025	0.260
Whole trial (25-56 d):	Weight gain (g/d)	49.1	49.5	5.01	0.668
	Feed intake (g/d)	124.7	124.1	13.7	0.953
	Feed efficiency (g gain/g intake)	0.396	0.400	0.021	0.331

[1]HNA = High sodium content (2.6 g/kg DM); LNA = Low sodium content (1.6 g/kg DM). [2]RMSE= Root means square error, number of replicates = 7 (ileal content samples pooled 2 by 2)

The results of our experiment indicate that is possible to reduce the present dietary sodium recommendations for fattening rabbits to 1.6 g/kg DM during the first two weeks after weaning, without impairing either the fattening performances or the ileal digestibility of DM and CP. In addition, this study indicates that the level of sodium in the diet affects the gut absorption of methionine and cystine and several non essential AA.

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