VERY LOW PROTEIN, AMINOACID-SUPPLIED DIET FOR HEAVY BROILER RABBITS: EFFECTS ON NITROGEN METABOLISM, AND DIGITAL EVALUATION OF EXCRETA AND PRODUCTS

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ABSTRACT

A very low protein diet (A, 15.9% DM) for the fattening of heavy broiler rabbits was supplied by the four available synthetic amino acid and compared to a control feed (C, 19.7% DM) in a study carried in metabolism cages with 12 (6+6) rabbits controlled at 81-84 days, then slaughtered at 87 days. The diets were anisoproteic, but isoenergetic and isofibrous. The low protein supply produced strong reduction in Blood Urea (-32%) and in Urea Urine (-37%) thus in the N-urinary emission (-29%; P<0.12). In the gastro-intestinal trait, however, N-feed was on average less utilized by the A group and refused (N faeces +20%; P<0.2). This findings appear to be biased because an exceptionally late growth in the Control group (50 vs. 40 g/d) provided by a push in glucose, unreal with respect to the growth trial (30.1 vs. 36.8 g/d) corroborated by favorable environmental conditions, resulting in a 29% higher N-retention for C a value clearly and strongly overestimated. Digital evaluation of fresh urine and faeces were able to discriminate the groups, while less or nothing the live, or the carcass or meat.

Key words: Rabbits, Protein, Amino acid, Metabolism, Urine, Feces, NIRS, Fluorescence spectroscopy, Electronic nose.

INTRODUCTION

Substantial improvement in nitrogen metabolism of growing pigs was recently achieved by using four synthetic Amino Acid (AA) (Rademacher**,** 2000). A switch into metabolic pathway of nitrogen allows a dramatic reduction in N-urine output, mainly composed of Urine-Urea-Nitrogen (UUN). A weak level of Blood-Urea-Nitrogen (BUN) is functional to this pattern and furthermore it is beneficial to homeostatic healthy conditions, also in rabbits. In fact (Saito and Hasegawa, 2003) in a japonais study with 198 bunnies, observed that the threshold of BUN 27 mg/dl unleashed risks of fatal enteritis at 27% with a global mortality rate of 49%. New digital rapid and cheap techniques, as NIRS and Fluorescence Spectroscopy (FS) or the Electronic Nose (EN) are now available to fingerprint some specific materials in animal production, with silages, milk or rabbit meat (Masoero *et al*., 2007) and finally, in the live skin of animal, as a truly paradigm of animal wellness (Masoero *et al*., 2005b). In a companion paper (Cherubini *et al*., 2008) we evaluated the performance of a very low protein diet supplied to essential AA for optimal, sub-maximum growth. In this second paper we analyzed several aspects of nitrogen metabolism, haematic parameters, clearence and the quality of the output excreta and of the meat products by using conventional and new available digital techniques.

Full application of the CE Directive 91/676 will probably have an impact also on large rabbit farms: therefore a more accurate estimation of the consequences of low protein diets on performances and nitrogen turnover in this species is the rational background of this preliminary experiment.

MATERIALS AND METHOD

Animals and analyses

Twelve *Bianca Italiana* rabbits were reared in metabolic cage since 31 to 84 days on two anisoproteic (19.7 vs. 15.9 CP%DM), isoenergetic and isofibrous diets, then slaughtered. Feeding was *ad libitum* by two *formulae*: a Control diet with normal protein level (C2, by Cherubini *et al*., 2008, Table 1) and a very low protein (A2) diet with supplemented AA.

According the common EU guidelines for digestibility trials (Perez *et al*., 1995) the 4-days control period started on Monday when rabbits were 81-days old. The faeces were dried in a ventilated oven at 60°C then grinded at 1-mm. An estimate of their chemical composition was obtained by previous NIRS equations. Urine fresh samples collected on the 86th day were analized by an IL device with two IL Tests ™ for Creatinine and Urea so the Urine-Creatinine (UCR) and the Urine-Urea (UU) transformed to Urine-Urea-Nitrogen (UUN) contents were determined. Blood samples were collected at slaughtering on the morning of the 87th day, without fasting. Clinical parameters were immediately determined by an i-STAT instrument (HESCA, Fort Collins, CO 80525, USA), to measure the following parameters: blood Creatinine (BCR), Na, K, Cl, urea, glucose, haematocrit (HCT), pH, partial pressure of carbonic anhydride (pCO_2), and to calculate: haemoglobin (HB), base excess (BE_{ecf}), difference in anions (An. Gap, = (Na + K) – (Cl + HCO_3)), total carbonic anhydride (TCO_2) and bicarbonate (HCO_3) (Masoero *et al*., 2005a). The clearance (ml urine/min) was calculated by the ratio of UCR to BCR concentrations weighed for the urine volume. The fresh samples of urine on the same morning were sequentially submitted to Fluorescence Spectroscopy (FS) (JASCO FP-6300: excitation 300 nm; emission 300-500 nm; 400 digits), to NIRS examination, by a fast portable UV-Vis-NIR device (Model LSP 350-2500 nm, LabSpec Pro, ASD, Analytical Spectral Devices Inc., Boulder, CO, USA) (350-2500 nm; 2151 digits) and to Electronic Nose (EN) evaluation (PEN-2 AIRSENSE, Analytics GmbH). Electronic nose evaluation of faeces was conducted on a 2-litres Nallophan bag air sample, aspirated by a portable vacuum-pump, onto the fresh daily-faeces placed in a 20 cm-Ø glass tunnel. Live rabbits were scanned by the same portable NIR Spectroscope on the loin region (Masoero *et al*., 2005b).

Statistical Analysis

Biological traits were analyzed using GLM SAS-procedure with a fixed effects model considering the two levels of the feeding factor. The digital measurements were submitted to a multivariate statistical evaluation performed by the Partial least squares (PLS) method, a chemometric tool which is well adapted to obtain a true synthesis of all available information underlying the electromagnetic or the aromatic "spectra". All the digits, were pre-treated as Standard Normal Variates with Detrend (SNVD), derived and then smoothed. The WinISI II software (Infrasoft International, Port Matilda, PA, USA) was chosen to perform the chemometrics, using a cross-validation system to assess the optimal number of latent, and allowing one passage for the elimination of any outliers (t>2; H>10). The olfactometric measurements of the ten MOS sensors registered for 30 sec were elaborated as contiguous arrays, in a set of 300 digits (Masoero *et al.* 2007). Binary discriminant contrasts were directly fitted then the coefficient of determination in cross validation (R^2_{val}) was used for the equation development.

RESULTS AND DISCUSSION

In spite of the absolute old age of the rabbits in the control period, the daily gain was relative high, and higher in the Control (40.4 vs. 51 respectively in the A and C: -21%; $P<0.05$; Table 1). The conversion in weight of the total feed intake (FDC, 4.69 vs. 3.87) and of the protein intake (PER, 1.60 vs. 1.56) was not statistically different. In the growth trial (Cherubini *et al*., 2008) the emerging situation for the second period (67-94 d) was opposite. In fact the A group grew more and transformed significantly better the feed and the protein (Daily Weight Gain: DWG 36.8a vs. 30.1b; FCI 3.87 vs. 5.02; PER

1.66a vs 1.12c). The trial was unique, but these results derive from the best environmental and health conditions present in the separate cell, where the contemporaneous metabolic trial was carried on with no death observed. According these results in metabolism cage, the C diet could have been declared more performance, but the intrinsic greater risk to enteritis linked to higher protein level, had vanish this potential when put into realistic herd sub-optimal conditions, where mortality raised to 44% in C vs. 8% in A. Nevertheless, some interesting points emerged in biological traits of blood and urine (Table2).

Table 1: Performance, N input / output relationships and digital analyses of excreta

		R^2_{model}	RMSE	C	A	Pr>F	A/C
Performance	Daily Weight Gain 81-84 d (g/d)	0.39	7.39	51.00	40.40	0.05	-21%
	Daily Feed Intake 81-84 d (g/d)	0.06	28.4	194.1	181.0	0.49	-7%
	Feed Conversio Ratio 81-84 d	0.14	1.13	3.87	4.69	0.29	21%
	Protein Efficiency Ratio 81-84 d	0.002	0.46	1.56	1.60	0.91	2%
N and Urea partial balance	N input (g/d) _Ing	0.50	0.75	5.39	4.06	0.02	-25%
	Fecal N Digestibility	0.78	0.04	0.762	0.610	0.00	-20%
	Fecal N (% DM) _FN%	0.41	0.23	2.05	2.39	0.046	17%
	Fecal N Output (g/d) _FONg	0.19	0.30	1.31	1.57	0.21	20%
	Total Urine (g/d) _UVOLg	0.04	69.85	187.40	213.60	0.57	14%
	N Urea in urine (g/d) _UONg	0.28	0.42	1.61	1.15	0.12	-29%
	Total N excreta _Ong	0.04	0.57	2.92	2.72	0.59	-7%
	N Retained (g/d) _RNg	0.70	0.42	2.47	1.34	0.00	-46%
	Retention (%)	0.56	0.06	0.46	0.33	0.01	-29%
Digital analyses		R2cal	R2val				
	Electronic nose evaluation of feces	0.72	0.49				
	Electronic nose evaluation of urine	0.96	0.48				
	Fluorescence spectroscopy of urine	0.60	0.22				
	UV-VIS-NIR Spectroscopy of urine	0.97	0.31				

Table 2: Biological traits of blood and urine

Biological parameters	R^2_{model}	RMSE	C	A	Pr>F	A/C
Urine Creatinine (mg/dl) - U_CREA	0.00	12.60	61.75	62.34	0.95	1%
Blood Creatinine (mg/dl) - B_CREA	0.01	0.12	0.73	0.74	0.86	2%
Clearance (ml/min) - CLEA	0.02	2.54	19.45	18.89	0.75	-3%
Blood Urea - BU	0.67	3.93	30.50	20.60	0.01	-32%
Urine Urea - UU	0.54	37.3	194.0	121.4	0.02	-37%
Glucose (mmol/l)	0.43	9.02	119.2	105.4	0.06	-12%
Na (mmol/l)	0.10	1.93	142.25	143.40	0.40	1%
K (mmol/l)	0.47	0.42	4.58	5.28	0.04	15%
Cl (mmol/l)	0.45	1.85	99.00	102.00	0.05	3%
TCO2 (mmol/l)	0.21	5.73	25.50	20.20	0.21	-21%
Haematocrit, Hct, (%)	0.29	4.46	25.75	30.80	0.14	20%
_pH	0.28	0.09	7.19	7.09	0.14	-1%
_pCO2 (mmHg)	0.05	15.72	62.75	68.90	0.58	10%
HCO3 (mmol/l)	0.08	3.92	23.55	21.46	0.45	-9%
BE_{ECF} (mmol/l)	0.17	4.29	-4.50	-8.00	0.26	78%
Haemoglobin HB (g/dl)	0.29	1.51	8.78	10.48	0.14	19%
Anionic Gap (mmol/l)	0.05	4.07	23.75	25.40	0.56	7%

The muscular catabolism measured by the creatinine level in blood and urine appeared as unaffected by the diet, but the muscular anabolism linked to urea cycle was strongly changed by the low protein level intake, resulting in urea reduction of some 32% in blood and of 37% in urine. Few data from a previous experiment (Masoero *et al*., 2005a) registered levels of blood urea at 51 mg/dl in summer vs. 35 (-40%) in winter and with an average setting of the outperforming herds at a level of 36 vs. 50. In this experiment the level 20 pertinent to A group, realized in summer conditions, appeared to be a sub-

optimal level for growth while an optimal level vs. the security threshold 27 quoted by Saito and Hasegawa (2003); on the other hand the level of 30 for thr C group appeared to be quite safe and more performing as it appears when the energetic status of the rabbit will be considered: in fact the glucose level in the blood was significantly raised in the Control group (119 vs. 105 mmol/l) and well accorded to the higher weight increment. The observed levels appeared to be inferior to the average values of 138 in rabbits of similar age. However the general metabolic conditions appeared to be more favourable in the A group because high disposal in K (+15%; P<0.04), Cl (+3%; P<0.05), Haematocrit (+20%; P<0.14) and Haemoglobin (+19%; P<0.14) with reduced pH values (-1%; P<0.14).

The N input/output relationships (Table 1) exhibited significant reduction of N intake into the A group (-25%; P<0.02), which N was also overall less efficiently retained in the body (-29%; P<0.01). The gross N output was very different by groups, because in front of an average increase of N faeces (+20%; P<0.21), caused by its higher N level of undigested protein (+23%; P<0.05), the urine N-Urea contents was decreased of some 29% (P<0.12), because of the average increase in daily volume (+14%) which nullified the advantage of the 39% reduction in the Urea content in urine of the A group, above reported. The differentiated pattern in protein feedstuff of the two diets was characterised by the lack of soybean (-5.2%) and peas (-4%) replaced by wheat milling (+6.8%), corn (+7.5%), but they also differ in the amount of protein coming from alfalfa, sunflower, straw and barley. The diets were *a priori* anisoproteic, but isoenergetic: the aniso-digestibility was envisaged but not at a so high level and could be re-investigated. Gidenne *et al*. (2002) with four groups at different digestible fibre/crude protein levels, observed a strong decrease of protein fecal digestibility coefficient with low protein diets, decreasing from 71% for the 17.7% CP to 61% for the 13.9% CP contents; those findings was obtained at their older age of 64-67 days: in this trial we observed the animals two weeks older than those, and the apparent digestibility was unrealistic high in the control group (74% vs. 61%).

Table 3: Digital analyses of organs and products

Digital analyses		R^2c	R^2cv
UV-VIS-NIR Spectroscopy	Live rabbits	0.40	0.07
	Carcass liver	0.96	0.11
	Carcass fat	0.43	-
	Carcass loin	0.24	-
	Carcass hindleg	0.45	0.07
	Carcass belly	0.27	0.03
	Longissimus dorsi ethanol preparation	0.78	0.24
	Fat ethanol preparation	0.40	0.09
	Belly ethanol preparation	0.46	-
Electronic nose –EN	Electronic nose evaluation of raw L.d muscle	0.05	0.08
	Electronic nose evaluation of cooked hindleg	0.13	0.02

- negative cross-validation

The rapid digital analyses (Table 1) carried on the fresh urine, enhanced R^2-discriminant values, considered in validation mode, not superior to the 0.54 R^2-value reported for the Urea in urine (Table 2), but increasing since 0.22 for the Fluorescence, to 0.31 for the NIRS till to 0.48 for the electronic nose evaluation, this last an interesting instrument able to well differentiate also the fresh faeces (0.49).

The digital analyses carried on the loin of the live rabbits by portable NIRS was not able to assess real differences in hair and skin of the two groups (Table 3: R^2 =0.07), and not even in the organs and tissues of the carcass, with a little exception for the ethanol preparation of the *Longissimus* muscle (0.24). Moreover no substantial differences were observed by the EN in fresh or cooked meat.

CONCLUSIONS

A supply of AA of a poor and low-protein diet, as compared to a different standard normal protein level, can stabilize the blood Urea and reduce the Urea concentration in urine. The comparison about N excretion reduction was limited -on a very short period- to a special Control diet, which very well resulted in outperforming rabbit at old age, not validated by on-field experiment, and would be confirmed by further experiments.

The metabolic results thus confirm the possibility of substantial modification in Urine contents and characteristics, without modification in carcass and meat quality. Further studies are requested to achieve a more comprehensive database on the possible impact of nutrition on nitrogen output of intensive rabbit farms.

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