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A METHOD TO ESTIMATE ENDOGENOUS LOSSES OF NITROGEN AND AMINO ACIDS AT ILEAL LEVEL IN GROWING RABBITS

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Abstract: Apparent ileal digestibility can be corrected to give the true ileal digestibility of the nitrogen and amino acids provided by the diet, by determining the flow of endogenous nitrogen and amino acids (from desquamated epithelial cells of gastrointestinal mucosa, mucins and digestive enzymes). This flow of nitrogen and amino acids has been studied in adult rabbits fitted with a T-cannula, but remains unknown for growing rabbits. The aim of this work was to propose a method to estimate endogenous nitrogen and amino acid losses in the ileum of growing rabbits slaughtered at 64 d of age from 20:00 h. For this purpose, two experiments were carried out. The first was performed with 10 weaned rabbits fed with a diet with casein as the only source of protein (whose ileal digestibility is 100%) and labelled with ytterbium. This experiment allowed us to identify the relationship between the ileal flow of endogenous nitrogen (IF_{EN}) and the dry matter intake in the last 24 h before slaughter (DMI), which fits the equation: IF_{EN} (mg/d)=5.99 DMI (g/d) +133; (R^2=0.778, residual standard deviation=138, P<0.001, n=10). The second experiment was carried out with 36 rabbits fed the same diet but without ytterbium, with whose ileal content 9 pools were constituted to determine the amino acid profile of endogenous nitrogen, which was found to be rich in glutamic acid, serine, aspartic acid, glycine, valine and threonine (15.97±1.33; 8.00±0.80; 7.06±0.72; 6.24±0.77; 5.48±0.51 and 4.97±0.47 g/16 g of N, respectively) and poor in methionine and histidine (1.05±0.06 and 1.34±0.16 g/16 g of N, respectively). Knowing the DMI of a certain growing rabbit in the 24 h prior to slaughter, the combined use of the equation and the amino acid profile obtained makes it possible to estimate the ileal endogenous losses of each amino acid.

Key Words: Ileal, nitrogen, amino acids, endogenous losses, growing rabbit.

INTRODUCTION

The Green Deal aims to make Europe the first climate-neutral continent by 2050. To achieve this goal, among other actions, the development of a more sustainable livestock sector, reducing its emissions as much as possible, is proposed (Peyraud and MacLeod, 2020). Carbon emissions associated with rabbit production are lower than in most livestock species, due to the greater presence of by-products in their feeds (about 50%; De Blas and Mateos, 2020) and their lower methane production (Misiukiewicz *et al.,* 2021). However, despite the contribution of caecal fermentation and caecotrophy, growing rabbits excrete more than two-thirds of ingested N (Xiccato and Trocino, 2020). Although emissions from rabbit manure are a priori lower than in other species as they are more solid, we must make every possible effort to improve the efficiency of N utilisation in this species, and thus reduce its content

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in their excreta. To achieve this goal, it is essential to precisely determine both rabbit protein requirements and the protein value of the ingredients, to reduce the N not assimilated and which ends up being excreted. The excess of non-assimilated dietary protein almost always entails an extra cost and the mentioned greater environmental impact (Koneswaran and Nierenberg, 2008).

In protein nutrition, the classic concepts of crude protein (CP; N×6.25) and total amino acids have been widely surpassed by new concepts. These new concepts are increasingly adjusted to the process of digestion and absorption of these nutrients. Thus, compared to faecal digestibility, calculated from the difference between intake and faecal excretion, one of the most important advances was ileal digestibility. Ileal digestibility was proposed to assess the amounts of N and amino acids absorbed in the small intestine, from the difference between intake and flow at the level of the terminal ileum. It is assumed that these amounts are the best estimate of the availability for the metabolism of the animal, as the interference of the microbiota of large intestine, which causes the faecal excretion of N and amino acids to be appreciably different from its ileal flow, is eliminated.

Apparent ileal digestibility, calculated as indicated above, leads to an underestimation of the digestible N and amino acid contents of the diet, because part of them in ileal digesta are not of dietary origin. This part is endogenous losses, that is, they come from the digestive tract itself or from its adjoining glands, mainly as constituents of desquamated epithelial cells, mucins and digestive enzymes. Estimating this endogenous component of the ileal flow allows us to correct the apparent ileal digestibility, obtaining the true ileal digestibility of N and amino acids provided by the diet.

To determine endogenous losses, animals are fed with N-free diets or with diets whose N is completely digestible in the small intestine (such as that from casein). In this line, García *et al.* (2004) stated that the use of N-free diets is inadvisable because they lead to abnormally low feed intake, and recommended the use of casein-based diets. In both types of diets, it can be assumed that all the N and amino acids present in the content of the terminal ileum are necessarily endogenous. However, the possibility must be considered that a small part of them is of dietary origin and, if necessary, make the appropriate correction. In addition, in the case of rabbits, the effect of caecotrophy on the composition of ileal content must be avoided or minimised.

This endogenous component has so far been studied with an animal model of adult rabbits fitted with a T-shaped cannula surgically placed in the terminal ileum (García *et al.,* 2004, 2005; Villamide *et al.,* 2013). But endogenous component has not been studied in growing rabbits. The T-shaped cannula model is not applicable to growing rabbits (4 to 9 wk old), due to the difficulty of implanting the cannula in recently weaned rabbits and because complete post-surgical recovery would only be achieved when the animals reached an age that is not very representative of growing rabbits.

The main aim of this work is to develop a method to determine the endogenous losses of N and amino acids at the ileal level in growing rabbits by using a casein-based diet, and thus learn the true ileal digestibility of these nutrients.

MATERIAL AND METHODS

The experimental protocols were approved by the Animal Welfare Ethics Committee of the Universitat Politècnica de València (authorisation code: 2018/VSC/PEA/0116) and carried out following the Spanish Royal Decree 53/2013 on the protection of animals used for scientific purposes (Boletín Oficial del Estado, 2013). Two experiments were carried out to determine the relationship between the ileal flow of endogenous nitrogen and feed intake, and to obtain the amino acid profile of the ileal endogenous nitrogen.

Experimental procedure

Experiment 1

A total of 10 weaned rabbits (28 d of age; 772±34 g of body weight) belonging to 5 different litters from the R line were used. The R line was obtained after two generations of random mating from a pool of animals of three commercial sire lines (Estany *et al.,* 1992) and then selected by the average daily gain in the growing period during 38 generations. At weaning, animals were housed under a cycle of 12 h light (from 06:00 to 18:00 h) and 12 h dark

in individual cages where water and experimental diet were provided *ad libitum*. The experimental diet was casein-based, to which 0.8% ytterbium-labelled alfalfa hay was added according to García *et al.* (1999). The ingredients and chemical composition of the experimental diet are shown in Table 1.

Feed intake was monitored weekly up to 63 d of age, as well as in the 24 h prior to slaughter. At 64 d of age, animals were slaughtered by intracardiac injection of sodium thiopental (75 mg/kg of body weight). Slaughter was conducted between 20:00 h to 21:00 h to minimise the influence of caecotrophy on the composition of the digestive content (Merino and Carabaño, 2003). Samples of ileal digesta were obtained from the distal part of the small intestine (around 30 cm before the ileo-caeco-colic valve), placed in Petri dishes and stored at –20°C until freeze-drying and grinding for analysis. With the surplus of the 10 samples of ileal digesta, a single pool was constituted.

Experiment 2

A total of 36 weaned rabbits (28 d of age) belonging to 6 different litters from the R line were used. At weaning, animals were housed under a cycle of 12 h light (from 06:00 to 18:00 h) and 12 h dark in individual cages where water and a commercial feed (Cunivita SD, Nanta, Meliana, Spain) were provided *ad libitum* until day 49. At day 49, another experimental casein-based diet, with the same formula as in Experiment 1 but without marker, was provided. Their ingredients and chemical composition are shown in Table 2. All the animals were slaughtered at 64 d of age between 20:00 to 23:00 h to obtain samples of ileal digesta, following the same procedure described in Experiment 1. After freeze-drying and grinding, 9 pools were created with samples of 4 animals in each one.

Chemical analysis

Casein-based diets and ileal digesta (10 individual samples from Experiment 1 and 9 pools from Experiment 2) were analysed following the Association of Official Analytical Chemists procedures (AOAC, 2002) for dry matter (DM) (934.01) and N (990.03, Dumas or combustion method).

The amino acids in the casein-based diet and ileal digesta from Experiment 2 were determined after acid hydrolysis with HCl 6N at 110°C for 23 h, as described by Bosch *et al.* (2006), using a Waters HPLC system (Milford, MA, USA) consisting of two pumps (Mod. 515, Waters), an autosampler (Mod. 717, Waters), a fluorescence detector (Mod. 474, Waters) and a temperature control module. Aminobutyric acid was added as an internal standard after hydrolysis. Amino acids were derivatised with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and separated with a C-18 reverse-phase column Waters Acc. Tag (150×3.9 mm). Methionine and cystine were determined separately as methionine sulphone and cysteic acid, respectively, after performic acid oxidation followed by acid hydrolysis.

Table 1: Ingredients and chemical composition of the diet (Experiment 1).

Ingredients (g/kg)		Chemical composition (g/kg dry matter, DM)	
Casein[1]	160	DM (g/kg)	921
Corn starch[2]	280	Crude protein	148
Citrus pectin[3]	100	Ytterbium (mg/kg DM)	5.07
Wheat straw	240		
Fibrous concentrate[4]	160		
Soybean oil	18		
Calcium carbonate	5		
Dicalcium phosphate	25		
Sodium chloride	5		
Sodium bicarbonate	2		
Vitamin/trace element mixture[5]	5		

[1] Armor Proteins, Saint Brice-enCoglès, France. [2] Tereos Starch and Sweeteners Iberia, Zaragoza, Spain. [3] Classic CU 201, Herbstreith & Fox KG, Neuenbürg, Germany. [4] Arbocel ®, Rettenmaier Ibérica, Barcelona, Spain. [5] L-511, Trouw Nutrition España, Tres Cantos, Spain: Contains per kg of feed:, Vitamin A: 12000 IU, Vitamin D3: 1080 IU, Vitamin E: 45 IU, Vitamin K3: 1.2 mg, Vitamin B1: 2 mg, Vitamin B2: 6 mg, Vitamin B6: 2 mg, Vitamin B12: 10 µg, Nicotinic acid: 20 mg, Calcium pantothenate: 20 mg, Pantothenic acid: 18.4 mg, Folic Acid: 5 mg, Biotin: 75 µg, Choline chloride: 260 mg, Manganese: 20 mg, Zinc: 75 mg, Copper: 14 mg, Iodine: 1.1 mg, Cobalt: 0.49 mg, Selenium: 0.06 mg, Iron: 78 mg, Robenidine: 60 mg.

Table 2: Ingredients and chemical composition of the diet (Experiment 2).

Ingredients (g/kg)		Chemical composition (g/kg DM)	
Casein[1]	160	Dry matter (g/kg)	920
Corn starch[2]	280	Crude protein	156
Citrus pectin[3]	100	Amino acids:	
Wheat straw	240	Alanine	4.19
Fibrous concentrate[4]	160	Arginine	5.26
Soybean oil	18	Aspartic acid	9.34
Calcium carbonate	5	Cystine	0.67
Dicalcium phosphate	25	Glutamic acid	30.7
Sodium chloride	5	Glycine	3.32
Sodium bicarbonate	2	Histidine	3.93
Vitamin/trace element mixture[5]	5	Isoleucine	7.11
		Leucine	14.1
		Lysine	10.1
		Methionine	4.26
		Phenylalanine	7.38
		Proline	15.2
		Serine	7.31
		Threonine	4.68
		Tyrosine	4.48
		Valine	9.56

[1] Armor Proteins, Saint Brice-enCoglès, France. [2] Tereos Starch and Sweeteners Iberia, Zaragoza, Spain. [3] CEAMPECTIN SS 4510, Ceamsa, Porriño, Spain. [4] Arbocel ®, Rettenmaier Ibérica, Barcelona, Spain. [5] L-511, Trouw Nutrition España, Tres Cantos, Spain: Contains per kg of feed:, Vitamin A: 12000 IU, Vitamin D3: 1080 IU, Vitamin E: 45 IU, Vitamin K3: 1.2 mg, Vitamin B1: 2 mg, Vitamin B2: 6 mg, Vitamin B6: 2 mg, Vitamin B12: 10 µg, Nicotinic acid: 20 mg, Calcium pantothenate: 20 mg, Pantothenic acid: 18.4 mg, Folic Acid: 5 mg, Biotin: 75 µg, Choline chloride: 260 mg, Manganese: 20 mg, Zinc: 75 mg, Copper: 14 mg, Iodine: 1.1 mg, Cobalt: 0.49 mg, Selenium: 0.06 mg, Iron: 78 mg, Robenidine: 60 mg.

In addition, ileal digesta (the single pool from Experiment 1 and the 9 pools from Experiment 2) was subjected to neutral-detergent extraction according to Mertens *et al.* (2002). The resulting neutral-detergent residues were analysed for DM and N, as well as for amino acids in the case of those from the ileal digesta of Experiment 2, following the methodology described above.

Ytterbium in the casein-based diet and ileal digesta from Experiment 1 was analysed according to García *et al*. (1999), using atomic absorption spectrometry (Smith-Hieftje 22, Thermo Jarrell Ash, Massachusetts, USA), with samples previously incinerated (550°C) and digested by boiling with a solution of 1.5 M HNO_3 and 0.05 M KCl.

Calculations

Experiment 1

Ileal flow of DM (IF_{DM}, g/d) was determined by the dilution technique of a marker, according to the following equation:

$$IF_{DM}=DMI\times Yb_d/Yb_i$$

where DMI is the DM intake in the 24 h before slaughter (g/d), Yb_d is the dietary ytterbium content (mg/kg DM), and Yb_i is the ytterbium content in the ileal digesta (mg/kg DM).

Ileal flow of N (IF_N, mg/d) was obtained with the equation:

$$IF_N=IF_{DM}\times N_i$$

where N_i is the N content in the ileal digesta (g/kg DM).

Likewise, ileal flow of N bound to the neutral-detergent residue (IF_{NDR-N}, mg/d) was calculated as the best possible estimation of the N of dietary origin (not endogenous) that reaches the terminal ileum, mainly provided by the cereal straw and the fibrous concentrate, through the equation:

$$IF_{NDR-N}=IF_{DM}\times NDR_i\times N_{NDR_i}/10^3$$

where NDR_i is the neutral-detergent residue content in the single pool of ileal digesta (g/kg DM), and is the N content in the neutral-detergent residue of the single pool of ileal digesta (g/kg NDR_i).

Consequently, the ileal flow of endogenous N (IF_{EN}, g/d) was obtained by the difference between IF_N and IF_{NDR-N}. A linear regression analysis of the IF_{EN} on DMI was performed.

Experiment 2

The endogenous N content in ileal digesta (EN, g/kg DM) was calculated using the equation:

$$EN=N_{i\text{-}NDR_i}\times N_{NDR_i}/10^3$$

where N_i is the N content in ileal digesta (g/kg DM), NDR_i is the neutral-detergent residue content in ileal digesta (g/kg DM), and is the N content in the neutral-detergent residue of the ileal digesta (g/kg NDR_i).

Similarly, the endogenous amino acid contents in ileal digesta (EAA, g/kg DM) were also obtained according to the following equation:

$$EAA=AA_{i\text{-}NDR_i}\times AA_{NDR_i}/10^3$$

where AA_i is the amino acid content in ileal digesta (g/kg DM), NDR_i is the neutral-detergent residue content in ileal digesta (g/kg DM), and is the amino acid content in the neutral-detergent residue of the ileal digesta (g/kg NDR_i).

Finally, the content of each amino acid was expressed as g/16 g of EN.

Statistical analysis

Linear relationship between the IF_{EN} and the DMI was analysed with the REG procedure of SAS software (version 9.4, SAS Institute Inc., Cary, NC).

RESULTS

Relationship between ileal flow of endogenous nitrogen and feed intake

Table 3 shows all the data obtained in Experiment 1, used to determine the IF_{EN}. On average, DMI in the last 24 h before slaughter at 64 d of age was 161.3±40.7 g/d, IF_{DM} was 98.1±26.2 g/d, and IF_{EN} was 1100±276 mg/d. Using this information, Figure 1 shows the relationship between the IF_{EN} and the DMI [IF_{EN} (mg/d)=5.99 DMI (g/d)+133; R^2=0.778, residual standard deviation (RSD)=138, P<0.001, n=10].

Amino acid profile of the ileal endogenous nitrogen

Table 4 shows the main results from Experiment 2, including the N and amino acid contents of the ileal digesta, its neutral-detergent residue and the endogenous nitrogen.

DISCUSSION

The regression equation to estimate IF_{EN} from the DMI obtained in the current study for growing rabbits was very similar to that obtained by Villamide *et al.* (2013) for adult rabbits fitted with T-shaped cannulas in the terminal ileum (IF_{EN} (mg/d)=5.97 DMI (g/d)–49, R^2=0.807; RSD=69, P<0.001, n=11), whose samples were collected during 1 h between 19:00 and 23:00 h. In the current study, ileal flow of the N of dietary origin at the ileal level was calculated from the N content in the neutral-detergent residue of the ileal digesta, whereas in the above cited study it was calculated from the N content in the neutral-detergent residue of the diet. Nevertheless, in both cases,

Table 3: Dry matter (DM) intake in the last 24 h before slaughter at 64 d (DMI), ytterbium content in ileal digesta (Yb_i), ileal flow of DM (IF_{DM}), N content in ileal digesta (N_i), ileal flow of N (IF_N), ileal flow of N bound to neutral-detergent residue (IF_{NDR-N}) and ileal flow of endogenous N (IF_{EN}) from Experiment 1.

Rabbit	DMI (g/d)	Yb_i (mg/kg DM)	IF_{DM} (g/d)	N_i (g/kg DM)	IF_N (mg/d)	IF_{NDR-N} (mg/d)	IF_{EN} (mg/d)
1	152.0	7.59	101.6	12.9	1308	195	1112
2	193.5	7.89	124.3	11.8	1473	239	1234
3	211.9	8.13	132.2	11.8	1561	254	1307
4	184.3	9.34	100.1	17.0	1698	193	1505
5	69.1	8.83	39.7	13.9	553	76	476
6	156.6	8.51	93.4	12.2	1143	180	964
7	165.9	10.50	80.1	14.6	1167	154	1012
8	124.4	7.25	87.0	13.9	1206	167	1039
9	188.9	8.15	117.5	13.0	1528	226	1302
10	165.9	8.02	104.9	11.9	1248	202	1046
Mean	161.3	8.42	98.1	13.3	1289	189	1100
Standard deviation	40.7	0.94	26.2	1.6	320	50	276

Ytterbium in the diet: 5.07 (mg/kg DM), neutral-detergent residue in the single pool of ileal digesta: 526 g/kg DM, N bound to the neutral-detergent residue in the single pool of ileal digesta: 3.66 g/kg neutral-detergent residue. SD: Standard deviation.

IF_{EN} increased by approximately 6 mg for each g that DMI increased, even though the average DMI was 71% higher in the current study (161 *vs.* 94 g/d). Differences observed on DMI were because the animals used in the study of Villamide *et al.* (2013) were adults in maintenance, with lower requirements than the growing rabbits at 64 d of age used in the current study, which were in full growth. On the other hand, fasting IF_{EN} could be estimated from this regression equation as 133 mg/d, and would correspond to the minimal basal endogenous losses.

Table 5 describes the experimental conditions and compares the amino acid profiles of the EN obtained in the current study and in those available in the literature. In general, regardless of methodological differences (animal model: adult rabbits or growing rabbits; type of diet: casein-based or N-free diets; caecotrophy: allowed or not; time of day when ileal digesta was sampled: morning and evening or evening-night), the sum of the endogenous amino acids represents 75-80% of EN×6.25. To explain this percentage of recovery, it should be noted that: i) as

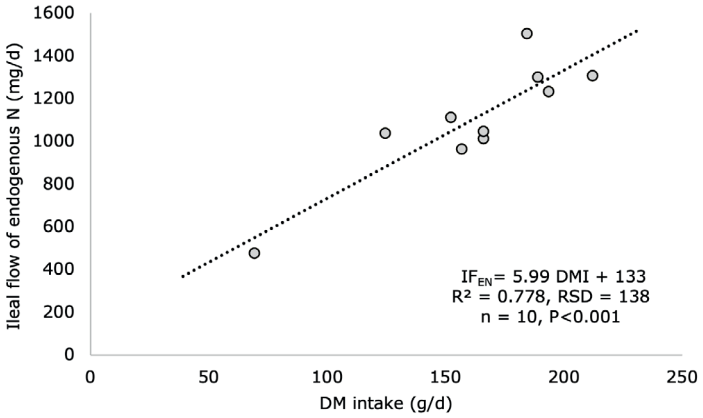

Figure 1: Relation between DM intake (DMI, g/d) and ileal flow of endogenous N (IF_{EN}, mg/d) at ileal level. RSD: Residual standard deviation.

Table 4: N and amino acid contents of the ileal digesta, its neutral-detergent residue and the endogenous nitrogen from Experiment 2 (mean±standard error).

	Ileal digesta (g/kg DM)	Neutral-detergent residue (g/kg)	Endogenous nitrogen	
			g/kg DM	g/16g of N
N	11.31±0.89	4.02±0.24	9.38±0.93	-
Essential amino acids				
Arginine	1.47±0.20	0.19±0.07	1.38±0.20	2.53±0.38
Cystine	1.01±0.08	0.18±0.03	0.93±0.07	1.72±0.23
Phenylalanine	1.41±0.09	0.34±0.01	1.25±0.09	2.29±0.22
Histidine	0.76±0.07	0.05±0.03	0.74±0.07	1.34±0.16
Isoleucine	2.94±0.17	0.39±0.01	2.76±0.18	4.97±0.49
Leucine	2.90±0.20	0.63±0.01	2.60±0.20	4.72±0.46
Lysine	1.85±0.14	0.37±0.01	1.68±0.14	3.04±0.26
Methionine	0.68±0.07	0.18±0.03	0.59±0.07	1.05±0.06
Threonine	2.98±0.17	0.44±0.02	2.77±0.17	4.97±0.47
Valine	3.32±0.20	0.60±0.02	3.03±0.20	5.48±0.51
Non-essential amino acids				
Aspartic Acid	4.27±0.32	0.75±0.02	3.91±0.33	7.06±0.72
Glutamic acid	9.46±0.50	1.22±0.07	8.88±0.52	15.97±1.33
Alanine	2.67±0.19	0.77±0.03	2.30±0.19	4.15±0.41
Glycine	3.61±0.25	0.56±0.04	3.34±0.26	6.24±0.77
Proline	2.90±0.15	0.71±0.02	2.56±0.15	4.64±0.44
Serine	4.76±0.30	0.58±0.03	4.49±0.31	8.00±0.80
Tyrosine	0.80±0.08	0.14±0.02	0.73±0.08	1.35±0.11

Neutral-detergent residue in the ileal digesta: 476±12 g/kg dry matter.

usual, the tryptophan content is not included, since its analysis is not well resolved; ii) factor 6.25 is used because it is assumed that all proteins contain 160 g N/kg, when actually this value is variable, and a different factor should be used for each protein; iii) it is assumed that all EN came from protein, when part of that nitrogen is in non-protein compounds such as nucleic acids.

In general, the endogenous amino acid profile of the ileal digesta is quite similar among the different studies. If we directly compare the results of the current study with those of Villamide *et al.* (2013) and García *et al.* (2004) when diets with casein were used and caecotrophy interference was avoided (since they were obtained with a quite similar methodology), it is observed that the most abundant amino acids are glutamic acid, serine, aspartic acid, glycine, valine and threonine, while the scarcest are methionine and histidine. The main difference between the results of the present study and those of Villamide *et al.* (2013) was that higher glutamic acid and serine contents, and lower tyrosine, phenylalanine and glycine contents in the ileal endogenous protein were observed in the present study. Compared to those of García *et al.* (2004), higher content of glutamic acid and lower content of arginine and cystine were observed in the present study.

Analogously, when compared to other species, Blok *et al.* (2017) and Adeola *et al.* (2016) pointed out that the ileal endogenous protein in chickens is rich in glutamic acid, aspartic acid, glycine, threonine, serine and proline, as well as poor in methionine and histidine, which closely reflects the composition of the mucoproteins. Likewise, Stein *et al.* (1999), point out that the endogenous protein in the ileum of growing pigs is rich in proline, glutamic acid, aspartic acid and glycine, as well as poor in methionine and histidine. All these studies reflect a very similar composition of the endogenous amino acid profile at both intra and interspecific levels.

Table 5: Comparison of the different works performed in rabbits where amino acid profile of ileal endogenous nitrogen was analysed (g/16g N).

	García *et al.* (2004)				Villamide *et al.* (2013)	Current study
Animal model	Cannulated rabbit does				Cannulated rabbit does	Slaughtered growing rabbits
Diet	Casein		N-free		Casein	Casein
Caecotrophy allowed	Yes	No	Yes	No	Yes	Yes
Ileal collection time	11:00	11:00	11:00	11:00	19:00	20:00
	20:00	20:00	20:00	20:00	23:00	23:00
Essential amino acids						
Arginine	4.63	4.08	4.31	4.85	3.63	2.53
Cystine	3.11	3.14	2.92	3.45	2.75	1.72
Phenylalanine	2.09	1.67	1.97	2.16	4.13	2.29
Histidine	1.53	1.58	1.42	1.65	1.29	1.34
Isoleucine	3.72	3.73	2.70	2.87	3.81	4.97
Leucine	4.77	4.45	3.92	4.57	4.31	4.72
Lysine	3.76	3.23	3.72	3.07	3.56	3.04
Methionine	0.96	0.87	0.98	0.87	0.81	1.05
Threonine	5.53	4.93	4.70	5.68	5.56	4.97
Valine	5.64	5.29	4.83	5.48	5.06	5.48
Non-essential amino acids						
Aspartic acid	7.53	6.97	6.85	7.56	7.22	7.06
Glutamic acid	12.66	12.57	8.60	8.61	12.47	15.97
Alanine	3.56	3.05	3.71	3.33	3.38	4.15
Glycine	5.18	6.05	5.65	10.51	8.00	6.24
Proline	5.41	4.83	3.38	4.91	4.66	4.64
Serine	6.45	6.59	4.38	4.47	5.75	8.00
Tyrosine	2.07	1.73	2.02	2.00	3.47	1.35
Sum of amino acids	78.6	74.8	66.1	76.0	79.8	79.5

CONCLUSIONS

The results of the present work allow us to propose a method to estimate the endogenous losses of nitrogen and amino acids at the ileal level in growing rabbits, in order to transform apparent ileal digestibility into true ileal digestibility values. The procedure to follow includes: i) using the equation obtained to estimate the ileal flow of endogenous nitrogen (IF_{EN}) from the DM intake in the last 24 hours before slaughter (DMI): IF_{NE} (mg/d)=5.99×DMI (g DM/d) + 133; and ii) estimating the ileal flow of each endogenous amino acid according to the amino acid profile of endogenous nitrogen presented in the results of this work.

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