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# Nitrogen and amino acid ileal and faecal digestibility of rabbit feeds predicted by an *in vitro* method

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ABSTRACT

Predicting the amino acid digestibility of ingredients is one of the main challenges in animal nutrition, due to its effect on animal performance, gut health and the environment. The aim of this work was to predict *in vivo* ileal and faecal digestibility of nitrogen and amino acids by using an *in vitro* method. Eleven ingredients (soybean meal 480, toasted full-fat soybean, sunflower meal 280 and 360, peas, wheat, wheat shorts, corn, gluten feed, dehydrated alfalfa and soybean hulls), commonly used in rabbit diets, were simultaneously evaluated *in vivo*, and subjected to a three step enzymatic *in vitro* digestion. Dry matter (DM), nitrogen (N) and amino acids (AA) were analysed in the residues in order to determine their *in vitro* digestibility ($ND_{iv}$, $AAD_{iv}$). The correlation among *in vivo* apparent faecal and ileal, and true ileal digestibilities (AFD, AID and TID, respectively) of N and AA of the ingredients and the corresponding *in vitro* values were analysed using a regression procedure. The average $ND_{iv}$ was 0.875 and ranged from 0.764 of soybean hulls to 0.946 of full-fat soybean, $AAD_{iv}$ showed similar ranges of variation and their average values varied with the AA (from GLY and CYS: 0.83 to GLU and ARG 0.92). Values of $ND_{iv}$ were higher than the corresponding *in vivo* values (0.21, 0.10 and 0.05 points as average for AID, AFD and TID, respectively), but the correlation among them was relatively high (averaged 0.77, 0.76 and 0.65, respectively). An average of 72% of the variation in AA ileal digestibility was explained by the $ND_{iv}$ variation, but the latter only explained 55% of AFD AA variation. The accuracy of the prediction was similar among units, its RSD averaged 0.074. Methionine was the limiting amino acid most precisely predicted, as opposed to CYS and THR, which were predicted with the lowest accuracy. In conclusion, $ND_{iv}$ can help to discriminate among feedstuffs that are more or less digestible at ileal level better than chemical analysis.



## 1. Introduction

Reducing the possible negative interactions between animal production and the environment is one of the main challenges when maintaining intensive animal rearing systems. The European Union produces about one third of the feed protein that it consumes (FEFAC, 2014) and the transport of soybean from South America to Europe makes up about 18% of the global

*Abbreviations:* AA, amino acids; $AAD_{iv}$, *in vitro* amino acids digestibility; AFD, apparent faecal digestibility; AID, apparent ileal digestibility; $D_{iv}$, *in vitro* digestibility; $DMD_{iv}$, *in vitro* DM digestibility; $ND_{iv}$, *in vitro* N digestibility; TID, true ileal digestibility.

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**Table 1**
Range of nitrogen composition (g/kg DM) and *in vivo* apparent faecal, apparent ileal and true ileal digestibilities of the 11 feedstuffs evaluated by Villamide et al. (2013).

| | Nutritional content (g/kg DM) | | | | Apparent faecal digestibility | | | | Apparent ileal digestibility | | | | True ileal digestibility | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | std | min | Max | Mean | std | min | max | Mean | std | min | max | Mean | std | min | max |
| Nitrogen | 44.1 | 21.9 | 19.0 | 86.4 | 0.778 | 0.10 | 0.65 | 0.96 | 0.664 | 0.17 | 0.31 | 0.87 | 0.820 | 0.11 | 0.53 | 0.93 |
| Essential amino acids | | | | | | | | | | | | | | | | |
| Arginine | 20.9 | 13.3 | 7.19 | 41.0 | 0.838 | 0.10 | 0.71 | 0.97 | 0.78 | 0.12 | 0.62 | 0.92 | 0.872 | 0.07 | 0.75 | 0.95 |
| Cystine | 5.2 | 2.36 | 2.42 | 9.1 | 0.581 | 0.18 | 0.35 | 0.79 | 0.301 | 0.33 | −0.22 | 0.73 | 0.532 | 0.24 | 0.18 | 0.87 |
| Histidine | 7.1 | 3.55 | 3.22 | 13.5 | 0.856 | 0.08 | 0.73 | 0.97 | 0.802 | 0.11 | 0.57 | 0.92 | 0.883 | 0.09 | 0.67 | 0.96 |
| Isoleucine | 12.5 | 7.18 | 4.91 | 25.8 | 0.805 | 0.10 | 0.67 | 0.95 | 0.78 | 0.13 | 0.52 | 0.93 | 0.922 | 0.10 | 0.67 | 0.98 |
| Leucine | 20.2 | 9.62 | 10.7 | 39.3 | 0.813 | 0.09 | 0.68 | 0.95 | 0.785 | 0.10 | 0.54 | 0.90 | 0.874 | 0.08 | 0.66 | 0.94 |
| Lysine | 14.1 | 8.50 | 4.23 | 31.9 | 0.817 | 0.10 | 0.64 | 0.96 | 0.795 | 0.11 | 0.62 | 0.93 | 0.915 | 0.07 | 0.73 | 0.98 |
| Methionine | 4.5 | 2.53 | 2.26 | 9.7 | 0.852 | 0.08 | 0.73 | 0.97 | 0.85 | 0.10 | 0.60 | 0.93 | 0.93 | 0.08 | 0.72 | 0.99 |
| Phenylalanine | 13.5 | 7.37 | 5.87 | 28.4 | 0.810 | 0.09 | 0.69 | 0.95 | 0.704 | 0.13 | 0.44 | 0.87 | 0.838 | 0.10 | 0.62 | 0.93 |
| Threonine | 10.2 | 5.38 | 4.11 | 20.1 | 0.688 | 0.16 | 0.42 | 0.92 | 0.536 | 0.23 | 0.16 | 0.82 | 0.776 | 0.13 | 0.49 | 0.91 |
| Valine | 15.3 | 7.71 | 6.72 | 28.3 | 0.793 | 0.10 | 0.67 | 0.95 | 0.724 | 0.14 | 0.43 | 0.89 | 0.87 | 0.10 | 0.61 | 0.95 |
| Non essential amino acids | | | | | | | | | | | | | | | | |
| Alanine | 13.7 | 5.78 | 6.43 | 23.3 | 0.788 | 0.10 | 0.67 | 0.93 | 0.77 | 0.11 | 0.52 | 0.89 | 0.873 | 0.08 | 0.66 | 0.94 |
| Aspartic acid | 28.6 | 18.28 | 9.73 | 63.6 | 0.820 | 0.11 | 0.66 | 0.97 | 0.76 | 0.12 | 0.57 | 0.92 | 0.885 | 0.08 | 0.69 | 0.95 |
| Glutamic acid | 47.1 | 25.27 | 21.00 | 90.6 | 0.845 | 0.09 | 0.70 | 0.96 | 0.781 | 0.15 | 0.48 | 0.94 | 0.897 | 0.10 | 0.65 | 0.98 |
| Glycine | 13.9 | 7.07 | 4.77 | 25.8 | 0.764 | 0.12 | 0.58 | 0.95 | 0.564 | 0.19 | 0.13 | 0.77 | 0.87 | 0.11 | 0.58 | 0.97 |
| Proline | 15.2 | 5.43 | 9.30 | 24.7 | 0.808 | 0.07 | 0.68 | 0.92 | 0.749 | 0.13 | 0.41 | 0.86 | 0.818 | 0.16 | 0.36 | 0.95 |
| Serine | 11.8 | 6.56 | 4.52 | 25.2 | 0.705 | 0.16 | 0.42 | 0.94 | 0.564 | 0.24 | 0.19 | 0.88 | 0.779 | 0.16 | 0.42 | 0.95 |
| Tyrosine | 9.5 | 5.14 | 4.34 | 20.1 | 0.719 | 0.14 | 0.52 | 0.93 | 0.578 | 0.17 | 0.30 | 0.84 | 0.738 | 0.13 | 0.47 | 0.90 |

warming potential of pork production overall (Reckmann et al., 2012). Therefore, an accurate estimation of amino acid bioavailability from a wide variety of protein sources could allow us to formulate feeds with a lower crude protein (CP) content, at lower cost than nowadays commercial feeds, without impairing performance, while decreasing our dependence on soybean meal.

An accurate estimation of the amino acid bioavailability in feeds is essential to formulate cheaper diets that are environmental friendly and especially in the case of rabbits, cause fewer digestive health problems. The ileum is the last segment of intestine where amino acids (AA) can be absorbed. In consequence, the true ileal digestibility of protein and amino acids of feedstuffs is the best estimation of the supply of these nutrients (García et al., 2004, 2005; Villamide et al., 2013) to meet the requirements for rabbit growth and to control their availability for microbial proliferation (Chamorro et al., 2007). Several experiments have been performed to evaluate different units and to characterize the protein value of feedstuffs usually included in rabbit diets (García et al., 2005; Villamide et al., 2013). These *in vivo* trials are very expensive, time consuming and may compromise the welfare of the rabbits since they need to be cannulated or slaughtered for ileal evaluation. Therefore, there is a need to use simpler, faster and cheaper methods for routine determination of protein and amino acid digestibility in feeds. An enzymatic *in vitro* method was developed for rabbits, based on that proposed by Boisen (1991) for pigs, showing good results for faecal dry matter and energy digestibility (Ramos et al., 1992; Ramos and Carabaño, 1996). However, the *in vitro* estimation was worse for the apparent faecal digestibility of protein (Villamide et al., 2009). Endogenous protein at ileal and faecal levels has a great influence on *in vivo* apparent digestibility values, whereas *in vitro* digestibility could better match the true protein and amino acids availability. In fact, a correction for endogenous losses was proposed by Boisen and Fernandez (1995), to estimate the apparent protein and AA digestibility by *in vitro* methods. A relatively good fit was obtained for AA apparent ileal digestibility of barley samples for pigs (Pujol and Torrallardona, 2007). Similarly, true AA ileal digestibility of different soybean meal samples for poultry were estimated by immobilized digestive enzyme assay, with excellent agreement ($r^2$ ranged from 0.73 to 0.91, Schasteen et al., 2007). However, for assaying different samples of animal proteins, the goodness of the fit was limited ($r^2$ from 0.09 to 0.41, Rochell et al., 2013) because of the low range of *in vivo* values. To our knowledge there are no studies that relate *in vitro* and *in vivo* ileal N and AA digestibilities for rabbit feeds, but we can expect good results because a wide range of the protein sources are used in commercial diets.

The aim of this work was to predict the *in vivo* ileal and faecal digestibility of nitrogen and amino acids of a wide range of feedstuffs used in rabbit feed formulation by using an *in vitro* method.

## 2. Material and methods

### 2.1. Feeds

Eleven feeds (soybean meal 480, toasted full-fat soybean, sunflower meal 280 and 360, peas, wheat, wheat shorts, corn, gluten feed, dehydrated alfalfa 16 and soybean hulls) that account for about 90% of N content in rabbit diets were used to perform the *in vitro* method. These feeds were simultaneously evaluated *in vivo* as the only nitrogen supply in semisynthetic