



## Organic matter and nitrogen balance in rabbit fattening and gaseous emissions during manure storage and simulated land application



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### ABSTRACT

Expansion in global rabbit populations and in the number of rabbits raised for consumption necessitates assessment of the environmental impact and sustainability of rabbit production systems. This study undertook two evaluations: utilization (animal efficiency) of organic matter (OM) and nitrogen (N) produced from feed during rabbit fattening, and emission of ammonia (NH<sub>3</sub>), carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) from rabbit manure during its storage and land application in laboratory-simulated conditions. Results demonstrated manure contained approximately 30 and 65% of the OM and N in the daily feed intake, respectively. Additionally, rabbit manure was shown to produce sizeable NH<sub>3</sub> and GHG emissions. Specifically, cumulative N losses from storage and subsequent land application averaged 32.4 (NH<sub>3</sub>) and 2.2% (N<sub>2</sub>O) of excreted TN; cumulative proportions of OM lost as CO<sub>2</sub> and CH<sub>4</sub> averaged 51.3 and 0.4%, respectively. Finally, while manure incorporation into the soil effectively abated NH<sub>3</sub> emissions, it also showed its potential to increase N<sub>2</sub>O losses, a potent greenhouse gas (GHG). Future research should focus on identifying appropriate emission mitigation measures. Accurate field-scale measurements are also needed to make data available for the development of a reliable national emission inventory of the investigated gases.

### 1. Introduction

Raising rabbits is an active industry in several countries. The United Nations Food and Agriculture Organization estimates that rabbit livestock production has grown an average of 2.6% in each of the past 10 years; approximately 770 million rabbits are now reared each year worldwide (FAO-STAT, 2016). Currently, Asia accounts for about 83% of global rabbit production. Europe represents 14% of the market, while Africa (2%) and the Americas (1%) follow distantly. Sixty-eight percent of the 107 million rabbits counted in Europe in 2014 were in Italy, which made it the top European producer and second world producer of rabbit meat (262,500 t in 2013) (FAO-STAT, 2016). As is true for other livestock sectors (e.g., cattle, pigs, poultry), about 80% of total rabbit production is concentrated in the north of Italy. However, rabbit fattening farms in Italy are distributed differently; 50% are located in the south, 30% in the central, and 20% in the north of the country (Filiou, 2015).

Livestock farming system intensification and specialization is widely recognized as a major cause of environmental pollution (FAO, 2013) mainly due to the increased amount of manure produced by animals per unit of utilized agricultural area. Two topics of major importance are

surface and groundwater nitrate contamination as a result of runoff and soil leaching, and atmospheric impacts from ammonia (NH<sub>3</sub>) and greenhouse gas (GHG) emissions, namely carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O) (Capri et al., 2009; Sanz-Cobena et al., 2017).

Ammonia and GHG production and release are influenced by the physical-chemical characteristics of the manure (especially pH, dry matter - DM, organic matter - OM, total nitrogen - TN, and total ammoniacal nitrogen - TAN), which in turn are determined primarily by feeding factors (such as feed intake and diet digestibility, protein content and solubility, fiber content and degradability, presence of anti-nutritional substances), and to a lesser extent by animal age, housing system, environmental conditions, and production stage (e.g., Monteny et al., 2001; Ndegwa et al., 2008; Gerber et al., 2013; Sheppard and Bittman, 2013). However, information on the nature of manure production and N excretion from rabbit production systems under specific conditions in Italy is scarce, especially from the fattening period. Similarly, even though ammonia and GHG emissions may originate from any or all stages of rabbit manure management (i.e., production, storage, and application), the relative share of NH<sub>3</sub> and GHG emissions associated with the rabbit sector has yet to be precisely determined due

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to a lack of data. Most available emission data refer to those released from buildings during rearing (Estellés et al., 2010a, b; Calvet et al., 2011; Méda et al., 2014). Only a few data on emissions from rabbit manure storage (Estellés et al., 2014) have been reported, whereas little or none exists on emissions from application to land.

What makes investigation and quantification of these N processes important at this time is the convergence of three facts. One, widespread expansion of the rabbit livestock farming and their increasing numbers make it necessary to assess the environmental impact in relation to the production, storage, and utilization of manure as a crop fertilizer. Two, information is available on NH<sub>3</sub> and GHG emissions from rabbit manure managements in estimates produced by the Italian national inventory from a combination of default emission factors and personal communications from researchers and sector experts (Valli and Condòr, 2011; ISPRA, 2016). Three, rabbits have a particular digestive strategy (coprophagy) that may affect hindgut fermentation and potentially, gaseous production from manure.

To this end, this study investigated two related processes of rabbit production. The first aim evaluated the efficiency of the use of OM and N during rabbit rearing and fattening under intensive conditions. The second explored, under controlled laboratory conditions, the NH<sub>3</sub>, CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions from rabbit manure during its storage and subsequent land application by analysing and comparing two manure managements: 1) storage followed by application to the soil surface (S + SA), and 2) storage followed by application to the soil surface with incorporation into the soil at six hours after surface application (S + MI).

Scenario S + SA represents the traditional and most common rabbit manure application practice, whereas S + MI represents a technique increasingly adopted by Italian farmers to reduce the environmental impacts of NH<sub>3</sub> emissions. This relatively new practice aligns with current national regulations (DL 152/2006. Legislative Decree April 3, 2006) that recommend incorporating solid manure into the soil within 24 h of broadcasting.

## 2. Materials and methods

All procedures involving animals were conducted according to Italian law (DL 146/2001. Legislative Decree March 26, 2001) governing animal welfare in scientific experiments.

### 2.1. Animals, their housing, and their diet

The manure was collected from a rabbit farm (Carmagnola, Torino, Italy; 44°53' N, 7°41' E, at an altitude of 240 m a.s.l.), in which 120 rabbits (weaned crossbred Grimaud × Hycrole, 35 days of age, 935 g live weight - LW) were reared under semi-controlled environmental conditions (temperature 23 ± 5 °C, light length > 8 h) in individual California type cages (30 × 30 × 40 cm; 0.12 m<sup>2</sup> head<sup>-1</sup>) for 55 days (90 d of age, 3275 g LW). The animals were fed a standard pelleted diet *ad libitum* (Table 1) and had free access to clean drinking water.

### 2.2. Preparation of samples for analyses

During a four-day period (at 42 to 45 days of age; Perez et al., 1995), feed intake was measured and recorded for each of 12 randomly selected rabbits (1:1 sex ratio) housed in individual metabolic cages that separately collected faeces and urine (Perez et al., 1995). Feed intake was measured by weighing daily the individual feed administration and the remnant feed. Losses of feed during the rearing period were considered negligible, as the pellet form of the feedstuff enabled the rabbits to feed efficiently and prevented feed falling from the manger to the wire-mesh floor of the cage. Faeces and urine samples were also collected from the same individuals during the same period. Each sample was placed in a two-layer plastic bag to prevent moisture loss, and immediately frozen at -20 °C. Each sample was then thawed,

**Table 1**

Chemical composition of experimental diet (alfalfa meal, 30%; barley meal, 20%; dried beet pulp, 15%; wheat bran, 20%; soybean meal, 6%; sunflower meal, 6%; and vitamin-mineral supplementation, 3%; n = 12).

	Mean	SEM
Dry matter (g 100 g <sup>-1</sup> )	89.97	0.20
Organic matter (g 100 g <sup>-1</sup> DM)	93.20	0.17
Crude protein (g 100 g <sup>-1</sup> DM)	17.77	0.12
Ether extract (g 100 g <sup>-1</sup> DM)	3.70	0.15
Neutral detergent fibre (g 100 g <sup>-1</sup> DM)	38.13	0.54
Acid detergent fibre (g 100 g <sup>-1</sup> DM)	20.90	0.85
Acid detergent lignin (g 100 g <sup>-1</sup> DM)	4.53	0.15
Acid insoluble ash (g 100 g <sup>-1</sup> DM)	0.73	0.23
Gross energy (MJ kg <sup>-1</sup> DM)	18.80	0.12

mixed thoroughly, pooled, and then ground in a homogenizer (Tecator, Herndon, VA). Representative sub-samples were then taken and weighed in an aluminium foil pan, dried in a draft oven at 80 °C to a constant weight, and then stored for later chemical analysis. The excreta (urine and faeces) produced by the same group of rabbits were also collected each day for six days (48–53 days of age) and maintained at 4 °C for the gaseous emission trials. Each individual rabbit raised in each cage represented one replicate.

### 2.3. Feed and manure pH measurement and chemical analysis

A Crison portable pH-meter (Crison Instruments, S.A., Alella, Spain) fitted with a spear-type, automatic temperature compensation electrode was used for all pH measures. Proximate composition analyses of the diet (Table 1) and faeces were performed on duplicate samples according to the following AOAC (2006) methods: preparation of analytical sample (950.02), dry matter (DM) content (934.01), OM (942.05), total nitrogen (TN) and crude protein (CP) content (984.13), total ammoniacal nitrogen (TAN) content (941.04), ether extract (EE) content (2003.05), neutral detergent fibre (NDF) content (2002.04), acid detergent fibre (ADF) and acid detergent lignin (ADL) content (973.18). The acid-insoluble ash (AIA) content of the diet and faeces was determined using the Van Keulen and Young (1977) method while gross energy (GE) was measured with an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany).

### 2.4. Determination of organic matter balance and digestibility

The organic matter balance was calculated via the general equation to estimate OM excretion:

$$\text{OM excretion} = \text{OM intake} - \text{OM digestible}$$

where: OM intake is the amount of OM contained in feed consumed during the entire raising period (kg), and OM digestible is the amount of OM (kg) calculated as follows:

$$\text{OM digestible} = \text{Feed Intake (kg)} \times \text{Feed OM\%} \times \text{Feed OM apparent digestibility \%}$$

in which: the indirect digestibility method (Furuichi and Takahashi, 1981) using AIA as an inert marker was employed to determine Feed OM apparent digestibility.

### 2.5. Nitrogen balance and crude protein digestibility

The nitrogen balance was calculated according to the final report of the European Commission Directorate General XI (ERM/AB-DLO, 1999) following the general equation:

$$\text{N excretion} = \text{N intake} - \text{N gain}$$

where: N excretion is the entire amount of N excreted by rabbits during

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