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Evaluation of poultry manure and goat cheese whey anaerobic co-digestion

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Abstract

Hen droppings (HD) and Goat Cheese Whey (GCW) are two difficult substrates to be treated by anaerobic digestion due to their characteristics; however, their co-digestion offers the possibility of successfully treating these substrates together. The goal of this study was to evaluate the anaerobic co-digestion of HD and GCW at laboratory scale in order to determine biogas potential and possible operational problems before extrapolating results to a full-scale biogas plant. The potential methane production of HD, GCW and a mixture of both substrates was studied in batch mode, whereas the co-digestion of the mixture of HD and GCW was also studied in semicontinuous mode in a continuously stirred tank reactor. Results showed that the addition of GCW to HD increased methane production compared to HD alone; however, GCW alone showed the highest methane potential. In semi-continuous mode, the mixture of GCW and HD showed high biogas and methane yields ($582.0\pm29.5 L_{biogas} kg VS^{-1}$ and $381.2\pm19.0 L_{CH4} kg VS^{-1}$, respectively), although intense foaming incidents occurred. The composition of both substrates is complementary for their co-digestion and it improved the energy yield of the process. However, the economic viability of a biogas plant of 30 kWe, designed for treating HD and GCW, would be economically feasible only with subsidies for the investment and in the low range of investment costs for small scale biogas plants. Additional keywords: anaerobic digestion; biogas; renewable energy; livestock waste; economic analysis.

Abbreviations used: BMP (biochemical methane potential); CODs (soluble chemical oxygen demand); CODt (total chemical oxygen demand); CSTR (continuously stirred tank reactor); CW (cheese whey); FA (free ammonia); GCW (goat cheese whey); HD (hen droppings); HRT (hydraulic retention time); IA (intermediate alkalinity); IRR (internal rate of return); NPV (net present value); OLR (organic loading rate); PA (partial alkalinity); TA (total alkalinity); TAN (total ammonia nitrogen); TKN (total Kjeldahl nitrogen); TS (total solids); VFA (volatile fatty acids); VS (volatile solids).

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Introduction

Anaerobic digestion is a well proven technology for waste recovery in the form of energy, biofertilizer and/or many other useful and valuable by-products (Kougias & Angelidaki, 2018). Anaerobic digestion, also known as biogas technology, is associated with the reduction of greenhouse gases in the livestock and agricultural sector, the recycling of nutrients for agriculture, the production of renewable energy and, in general, the protection of the environment. All this, promoting the circular economy in the primary and agroindustry sector. Biogas technology is present all over the world covering from high industrialized plants (Poeschl *et al.*, 2010), such as the used in Europe, to low-tech, low-cost biogas plants widespread in developing countries (SNV & FACT Foundation, 2014). In either case, the use of local, sustainable, high-energy content substrates is essential to bring technology wherever is not present, adapting to the reality of each place.

Animal manures and agro-industrial by-products have been used in anaerobic digestion for many years, the former being the substrates mostly used to produce biogas. Animal manures are commonly codigested with other substrates in order to improve their digestion process, mainly focusing on increasing biogas production, reducing N concentration to decrease risks of ammonia inhibition, and improving chemical and physical characteristics to favor anaerobic degradation (Weiland, 2010). Specifically, hen droppings (HD) are a good example of complex substrate to be used in anaerobic digestion. It has as a high N content, which leads during the digestion process to inhibition due to excess ammonia concentration (Molaey et al., 2018), a high solids concentration, which makes difficult the use of the wet anaerobic digestion technology (Marchioro et al., 2018), a high content of feathers, which increases the risk of the formation of a superficial crust inside the digester and, moreover, the precipitation of calcium carbonate during the digestion process and substrate feeding could lead to the loss of working volume of the digester, ultimately reducing the efficiency of the process. Probably, the most important problem is the high N content, and therefore, it has been widely studied (Niu et al., 2013; Molaey et al., 2018). Different techniques are used in order to reduce N concentration of HD, such as membrane separation (Bayrakdar et al., 2017). But, probably, the simplest and most efficient (in economic and energy terms) technique for avoiding ammonia inhibition issues in the digestion of HD is the co-digestion with high C content substrates.

On the other hand, cheese whey (CW) is an agroindustrial by-product with a high availability, produced from the cheese manufacturing. Two different types of CW are produced depending on the casein precipitation method used for cheese production: acidic whey (pH<5) and sweet whey (pH=6-7) (Carvalho et al., 2013; Carlini et al., 2015). Moreover, CW composition depends on the manufacturing process and the milk type (cow, goat, sheep, etc.) (Carvalho et al., 2013). In any case, CW has a high content of lactose (39-60 kg m⁻³), fats (0.99-10.58 kg m⁻³) proteins (1.4-8.0 kg m⁻³) and mineral salts (4.6-100 kg m⁻³), with chemical oxygen demand (COD) values ranging from 50-102 kg m⁻³, being highly biodegradable and with a low alkalinity (Prazeres et al., 2012). Therefore, any form of CW has a rich nutritional value, but also, a high pollution potential (Chatzipaschali & Stamatis, 2012; Prazeres et al., 2012). Because of its high biodegradability, anaerobic digestion has been widely studied as a convenient process for CW treatment and valorization (Yan et al., 1988; Beszédes et al., 2010). Despite its high specific biogas yield (Rico et al., 2014; Escalante et al., 2018), anaerobic digestion of CW also showed lack of alkalinity and quick acidification (Lo & Liao, 1986; Hublin et al. 2012). Therefore, its co-digestion with substrates which provide alkalinity, such as HD, is a suitable solution for valorizing CW into biogas.

Nowadays, the production of CW is a problem for worldwide small and medium scale cheese producers who cannot find an affordable solution for its treatment and management (Prazeres et al., 2012; Carvalho et al., 2013; Escalante et al., 2018). Specifically, farmers in the Canary Islands produce high quantities of goat cheese whey (GCW), a large part of it being produced in small and medium scale artisan cheese factories. Dupuis (2015) estimated CW production in the Canary Islands to be between 70,000 and 90,000 m³ per year, being most of it GCW (≈86%) and most of cheese producers in the Canary Islands small, artisan producers. Only a small fraction of the CW produced in the Canary Islands is used for feeding animals and for agricultural purposes, most of it being discharged into the sewage system or disposed without any treatment in the environment, being both options forbidden and with negative effects to the environment and to the local water treatment systems. These practices can entail legal problems for farmers.

Availability of GCW for anaerobic digestion is a great opportunity for cheese manufacturers, livestock farmers and the agricultural biogas industry, which is currently inexistent in the Canary Islands (Gobierno de Canarias, 2017). On the one hand, there is a great potential for GCW producers for treating adequately this by-product through anaerobic co-digestion; on the other hand, livestock farmers could use GCW as cosubstrate for improving biogas production from animal manures, increasing profitability of biogas plants due to better economics of scale and higher energy production.

This study focuses on finding a suitable solution for the treatment of HD produced in a laying hen farm in Fuerteventura island (Canary Islands) with around 16,000 laying hens. The goal of the study is to evaluate the anaerobic co-digestion of HD and GCW at laboratory scale in order to determine biogas potential and possible operational problems to extrapolate results to a full-scale biogas plant producing renewable energy for the farm.

Material and methods

Substrates and inoculum

Two substrates were used during this study: HD and GCW. HD samples were collected from two different farms in the Canary Islands (Spain), one located in Puerto del Rosario (Fuerteventura) and the second one in Arico (Tenerife). Both farms use the conventional cage rearing system for laying hens, droppings being collected on conveyor belts and being automatically transported to the dunghill located outside the rearing

building. HD samples were collected directly from the conveyor belts to ensure freshness. Afterwards, samples were kept in the fridge at -20 °C until they were used in the experiment. This procedure was repeated three times throughout this research, two times for the farm located in Fuerteventura (sample 1 and sample 2) and one time for the farm located in Tenerife (sample 3). The aim of collecting different samples throughout the research was to use mainly fresh samples, avoiding the need to store samples for long time in the freezer. Samples were analyzed in total solids (TS) and volatile solids (VS) before freezing.

GCW samples were collected from two different goat farms that produce their own artisan cheese, one located in Adeje (Tenerife) and the other one in Fasnia (Tenerife). Each time fresh samples were collected, they were transported to the laboratory and frozen as quickly as possible to avoid degradation until their use in the experiment. Before freezing the samples, a subsample was analyzed in TS, VS and pH. This procedure was repeated two times throughout this research, one time in each farm. A subsample of the first sample was also freeze-dried before analyzing its ultimate composition (C, H, N, S).

Anaerobic biomass (inoculum) used for the different experiments was collected from a biogas plant located in Gran Canaria which co-digests Organic Fraction of Municipal Solid Waste (OFMSW) and wastewater sludge, working at 37°C. Inoculum was pre-digested before its use in the anaerobic assays in order to reduce endogenous biogas potential. TS and VS concentration were 51.2 g L⁻¹ and 35.6 g L⁻¹, respectively. Total Kjeldahl Nitrogen (TKN) concentration was 6.7 g L⁻¹ and total ammonia concentration was 1.4 g L⁻¹. Total Chemical Oxygen Demand (CODt) and Soluble Chemical Oxygen Demand (CODs) were 80,650 mgO₂ L⁻¹ and 10,970 mgO₂ L⁻¹, respectively, with an Intermediate Alkalinity (IA)/Partial Alkalinity (PA) ratio under 0.3 (0.14), corresponding to a PA of 12,636 mgCaCO₃ L⁻¹ and an IA of 1,795 mgCaCO₃ L⁻¹. Finally, pH was 8.4.

Biochemical methane potential (BMP) assays

BMP assays were carried out according to the guideline VDI 4630 (VDI, 2006). HD, GCW and a mixture of both substrates were analyzed (see Table 1). Substrates were placed into 500 mL reactors with the corresponding amount of inoculum according to the VDI 4630 (VS substrate /VS inoculum ≤ 0.5).

HD and the mixture of HD and GCW were analyzed in triplicate, whereas GCW was analyzed in duplicate. The substrates were crushed in a conventional crusher before being introduced into the reactors. Blank reactors were run in duplicate only with inoculum for the determination of endogenous methane production. Table 1 shows the content of each reactor of the assay. Reactors containing HD and the mixture of HD and GCW were diluted with water in order to have the same TS concentration in each of the reactors. All reactors were tightly closed and submerged into water kept at 37 °C by a thermostatic bath. Content of reactors was manually stirred once a day. The duration of the test was 56 days.

Methane production was measured using 500 mL inverted transparent plastic graduated cylinders, which were re-filled when necessary. Cylinders were filled with sodium hydroxide (NaOH) at 2.5% (w/w) in order to dissolve carbon dioxide and measure only methane production.

Ultimate analyses of substrates were performed before blending them in order to balance C/N ratio of the mixture of HD and GCW. Analyses of TS, VS, CODt, CODs, TKN, PA, total alkalinity (TA) and pH, were performed at the beginning and at the end of the assay.

Semi-continuous assay

The mixture of HD and GCW was co-digested in semi-continuous mode in a Continuously Stirred Tank Reactor (CSTR) of 12 L working volume. The composition of the mixture was such that the C/N ratio was at least 10, considered the minimum value in order to

Table 1. Content of each substrate (GCW=goat cheese whey; HD = hen droppings) in each of the reactors used in the BMP assays and estimated C/N ratio.

	Inoculum (mL)	GCW (g)	GCW (g VS)	HD (g)	HD (g VS)	Water (g)	C/N ratio
Reactors 1,2,3	350	0	0	24.3	6.95	90.8	7.2
Reactors 4,5	350	190.6	6.95	0	0	0	21.6
Reactors 6,7,8	350	43.7	2.77	14.6	4.17	55.0	10.3
Reactors 9,10	450	0	0	0	0	0	-

VS = volatile solids.

avoid ammonia toxicity during the digestion process. The mixture of substrates for feeding the reactor was done several times throughout the experiment to work with fresh substrates. This led to different composition of the mixtures (see Table 2).

The reactor was started by placing inside 12 L of inoculum. Afterwards, reactor was closed, and gas tightness was verified. CSTR was kept at constant temperature in the mesophilic range (37 °C) by water heated by a thermostatic bath and pumped around the reactor through a fabricated plastic casing. The content of the reactor was stirred 15 minutes each two hours with an electrical mixer. The reactor was kept without feeding for one week, in order to acclimate inoculum to the new conditions of temperature. During this week gas production was not monitored, and therefore, the start of the semi-continuous assay was considered to be the first day of feeding.

Duration of the experiment was 60 days, during which different samples were collected from farms and analyzed each time in TS and VS. Organic Loading Rate (OLR) was low due to operational problems occurred at the beginning of the experiment, related to the characteristics of the substrates used (see section *Results* for more information). Consequently, Hydraulic Retention Time (HRT) was kept at 60 days after the startup period in order to stabilize the reactor. Feed and discharge of reactors were done manually every day.

Reactors were monitored by means of chemical and physical analysis of the digestate and biogas production and composition. pH, TS and VS were measured once a week, whereas CODt, CODs, PA, TA, TKN and total ammonia nitrogen (TAN) were measured every two weeks. Volume of biogas was monitored continuously using Milligascounters[®] (Dr. Ing. Ritter Apparatebau GMBH & Co. KG; Bochum, Germany). Biogas composition (CH₄, CO₂, H₂S and O₂) was measured approximately every two weeks directly from the reactor or from a gasometer.

Energy availability

The energy available for the use of the laying hen farm was determined based on the results obtained during the semi-continuous assay co-digesting GCW and HD. The following equations were used:

$$V_{CH4} = V_{mixture} \cdot VS \cdot Y_{CH4} \tag{1}$$

$$P_{electricity} = V_{CH4} \cdot LHV_{CH4} \cdot \gamma_{CHP_e}$$
(2)

$$P_{heating} = V_{CH4} \cdot LHV_{CH4} \cdot \gamma_{CHP_h}$$
(3)

where V_{CH4} is the potential daily methane production (m³ d⁻¹), $V_{mixture}$ is the daily volume of the mixture of GCW and HD fed into the digester (m³), VS is the content of volatile solids of the mixture (kg VS m⁻³), Y_{CH4} is the methane yield obtained during the semicontinuous assay (m³ kg VS⁻¹), $P_{electricity}$ is the electricity potential (kWh d⁻¹), LHV_{CH4} is the lower heating value of methane (kWh m⁻³), which is 9.96 kWh m⁻³, γ_{CHP_e} is the electrical efficiency of a Combined Heat and Power (CHP) unit, which was considered to be 30%, $P_{heating}$ is the heating potential (kWh d⁻¹) and γ_{CHP_h} is the heating efficiency of a CHP unit, which was considered 60% in this study.

Afterwards, an economic feasibility study was performed for this case. Several assumptions were considered for performing the economic feasibility study in order to get representative and useful results. Investment costs were considered for two different cases: Case 1=5,000 \in kWe⁻¹; Case 2=9,000 \in kWe⁻¹ (Deublein & Steinhauser, 2008; Scheftelowitz & Thrän, 2016) to provide results for low and high cost biogas plants. In the Canary Islands, where the laying hen farm is located, there are subsidies from the regional Government that farmers can use for the construction of biogas plants. These subsidies can reach 45% of the investment costs of a renewable energy facility (Gobierno de Canarias, 2018). Therefore, for the

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Μ	%HD	%GCW	TS (%)	VS (%TS)	CODt (gO ₂ L ⁻¹)	CODs (gO ₂ L ⁻¹)	TKN (g L ⁻¹)	TAN (g L ⁻¹)	рН	Days	C/N
M1	25	75	14.8	77.8	260.0	> 50.0	8.89	1.99	4.9	1-3	10.2
M2	25	75	15.3	74.5	185.8	> 50.0	8.70	1.91	4.9	4-17	10.2
M3	36	64	13.2	71.6	254.5	> 50.0	8.61	3.11	5.2	18-23	10.1
M4	36	64	11.7	76.7	306.5	> 50.0	7.98	3.25	5.1	24-50	10.1
M5	30	70	12.2	78.8	162.3	> 50.0	7.70	1.99	5.0	51-61	10.2

Table 2. Composition of the mixtures used during the semi-continuous assay.

M= number of mixture; %HD = amount of hen droppings (w/w in fresh) in the mixture; %GCW = amount of goat cheese whey (w/w in fresh) in the mixture; TS = total solids; VS = volatile solids; CODt = total chemical oxygen demand; CODs = soluble chemical oxygen demand; TKN = total Kjeldahl nitrogen; TAN = total ammonia nitrogen; Days = days during which each mixture was used; C/N = estimated C/N ratio of the mixtures based on the analyses of the first samples.

economic feasibility study 45% of the investment costs were obtained from these subsidies. Operating and maintenance costs must be considered when evaluating the economic feasibility of a biogas plant. This figure was considered to be 5% of the investment costs each year (Klavon et al., 2013). In order to evaluate incomes provided by the biogas plant, the real electricity demand and cost of electricity of the farm during a one-year period was considered. According to the data provided by the laying hen farm, average electricity cost for the farm is 0.16367 \in kWh⁻¹, with an average electricity consumption of 136.9 kWh d-1 (49,954 kWh year-1). Electricity production from biogas that exceeded electricity demand of the farm was considered to be sold to the electricity network at an average price 0.05729 € kWh⁻¹, which was the average price of the electricity pool in Spain during 2018 (Omie, 2018). No income was considered due to heat nor digestate selling. Finally, a lifespan of 20 years was considered for the biogas plant, with a discount rate of 6%. Economic parameters were used to determine the economic feasibility of such a project. The Net Present Value (NPV) describes the value at present time of the cash flows that a project (or an investment) will produce through its life cycle, and it is calculated according to equation (4). The Internal Rate of Return (IRR) is the discount rate that would make the NPV of the project equal to zero, and it is calculated according to equation (5).

$$NPV = \sum_{n=0}^{t} \frac{CF_n}{(1+r)^n} \cdot$$
(4)

$$\sum_{n=0}^{t} \frac{CF_n}{(1+IRR)^n} = 0 \tag{5}$$

where CF_n is the cash flow of year $n \in (\cdot)$, r is the discount rate (-) and t is the lifespan of the project.

Analytical methods

Ultimate analysis (C, H, N, S) were carried out by combustion using a CHNS Flash EA 1112 Organic Elemental Analyzer. The pH analyses were performed using a Crison pH sensor submerged directly into the reactors or samples. The pH of HD was analyzed after dilution with water at a ratio 1:5 (substrate:water). TS and VS were analyzed according to APHA (1992). PA and TA were determined by titration to pH 5.75 and 4.3, respectively, according to Ripley *et al.* (1986). IA is calculated as the difference between TA and PA, and it approximates volatile acids concentration in the reactor (Ripley *et al.*, 1986). CODt and CODs were analyzed by an adaptation of the 410.4 method of U.S. EPA, using a multiparametric photometer HI83399 (Hanna Instruments; Woonsocket U.S.A.). TKN was determined by the Kjeldahl method and TAN was analyzed with the same method but without the acid digestion step. Free ammonia (FA), as unionized ammonia (NH₃), was calculated using TAN concentration considering the equilibrium between NH_4^+ and NH_3 inside reactors, which depends on temperature and pH (Hansen *et al.*, 1998).

Gas analysis during the semi-continuous assay was performed with a Multitec 545 (Hermann Sewerin GmbH, Gütersloh, Germany) which has infrared sensors for measuring methane (0-100%) and carbon dioxide (0-100%) and electrochemical sensors to measure hydrogen sulfide (0-5000 ppm) and oxygen (0-25%).

Statistical analysis

Statistical analyses were performed in order to determine significant differences between samples used in the BMP test. Two parameters were evaluated: methane yield (L_{CH4} kgVS⁻¹) and VS removal (%). For parameters which population was normally distributed and with homogeneity of variance (VS removal) an ANOVA analysis followed by a HSD Tukey posthoc analysis was performed. For parameters which population was not normally distributed (methane yield) a non-parametric test was used (Kruskal-Wallis) followed by Mann-Whitney U test comparing groups by pairs. A significance level of 0.05 was used in all tests.

Results

Substrate characterization

Results of substrates characterization are shown in Table S1 [suppl.]. HD showed a pH close to neutral, a high TS content (between 24.2 and 38.6%TS) and a VS content between 65.4 and 74.3% (% d.m.). On the other hand, ultimate analysis of the first sample showed a high C content together with a high N content, leading to a C/N ratio of 7.18. Samples showed a low variability of organic matter content [between 74.3 \pm 1.4 and 65.4 \pm 0.2% VS (in TS)], whereas sample 1 showed a TS content higher than sample 2 and sample 3, probably, due to the place of collection in the farm and the different freshness of the samples at the time of collection, which can be affected by the accumulation time of the droppings on the belt.

In contrast, GCW showed high water content (> 90%) with a high portion of dry matter being organic matter (between 78.0 and 92.7%). pH was acid, with values around 5. Ultimate analysis of the first sample

of GCW showed a high C content (45.95%) and a low N content (2.13%), leading to a C/N ratio of 21.6. It should be noted that the GCW showed a constant and significant biological activity, which made impossible an appropriate ultimate analysis. Consequently, the sample was lyophilized before its analysis.

Both, for HD and for GCW, ultimate analysis of the first sample was supposed to be similar for the subsequent samples collected for this study due to lack of resources to perform more analysis.

Biochemical methane potential (BMP) assays

Figure 1.a shows the cumulative methane production during the BMP assay. At the beginning of the experiment there was a large delay in the production of biogas of 10-15 days. Afterwards, all reactors showed typical biogas production curves, with an exponential period of biogas production longer for GCW than for any other substrate. In fact, GCW showed the highest methane production at the end of the assay with $5.23 \pm 0.23 L_{CH4}$, followed by the mixture of GCW and HD with $2.78 \pm 0.02 L_{CH4}$ and HD alone with $2.26 \pm 0.02 L_{CH4}$ (results expressed as 'mean value \pm standard error').

GCW also showed the highest methane yield, in terms of L_{CH4} kg VS⁻¹, with 691.9 ± 42.1 L_{CH4} kg VS⁻¹, followed by the mixture of GCW and HD (357.4 ± 2.9 L_{CH4} kg VS⁻¹) and, finally, HD as sole substrate (282.1 ± 3.98 L_{CH4} kg VS⁻¹). The co-digestion of GCW and HD yielded higher methane than HD as sole substrate, increasing methane yield by 26.7% The Kruskal-Wallis analysis showed ($p=0.042 \leq$ 0.05) that there is at least one group which differs from the others. Therefore, the Mann-Whitney U test was performed, showing that HD and the mixture of GCW and HD are different (p=0.046 < 0.05). On the other hand, GCW, despite showing the largest methane yield compared to HD and the mixture of GCW and HD, showed no significant difference with HD (p=0.083 > 0.05) nor with the mixture of GCW and HD (0.076 > 0.05).

Regarding biodegradability, in terms of TS and VS, results followed the trend of methane production, with the highest biodegradability for the GCW (56.9 \pm 0.8% TS and 68.4 \pm 0.8% VS reduction) and the lowest for HD (37.6 \pm 1.0% TS and 39.7 \pm 0.6% VS reduction) with intermediate value for the mixture of both substrates (44.3 \pm 0.4% TS and 49.2 \pm 1.0% VS reduction). In this case and unlike methane production, results are much more even among the different samples analyzed. However, ANOVA analysis showed significant differences between samples (*p*=0.00001 <0.05) and HSD Tukey post-hoc analysis showed that all samples differed from each other in terms of organic matter removal (in all cases *p*<0.05).

Semi-continuous assay

The CSTR reactor was operated for 60 days, during which the mixture of HD and GCW was done several times in order to work with fresh substrates. Due to the different compositions of the samples of HD and GCW collected (see Table S1 [suppl.]), proportion of each substrate in the mixture was different throughout the experiment, looking for keeping a C/N ratio > 10. Table 2 shows composition of the mixtures used throughout the semi-continuous assay.

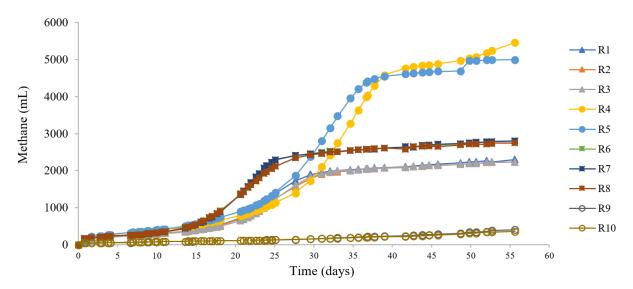


Figure 1. Cumulative methane production for each reactor: (\blacktriangle) hen droppings (HD), (\bullet) goat cheese whey (GCW); (\blacksquare) mixture of HD and GCW, (\circ) inoculum.

The composition of the mixtures showed differences, an aspect to take into account when analyzing results as this could influence the anaerobic digestion process. All mixtures showed a high TS concentration (> 11%), leading to a high organic load. Moreover, there were some characteristics that should be highlighted. The pH was acid, with values around 5. CODs/CODt ratio was high, between 16% and 30%, showing a high fraction of organic matter easily degradable. Finally, N content of the mixtures was between 7.7-8.9 g L⁻¹, despite the high amount of GCW used in the mixture.

Figure 2a shows digester operational conditions throughout the study, together with the periods during which the different mixtures were fed into the digester. Three different phases have been identified during the operation of the digester according to operational parameters and biogas and methane production (Figure 2b).

Phase 1 was the start of the experiment, that lasted 23 days. At the beginning HRT and OLR was fixed at 45 days and 2.5 kg VS m⁻³ d⁻¹. However, foam production inside the digester was high, leading to overpressure,

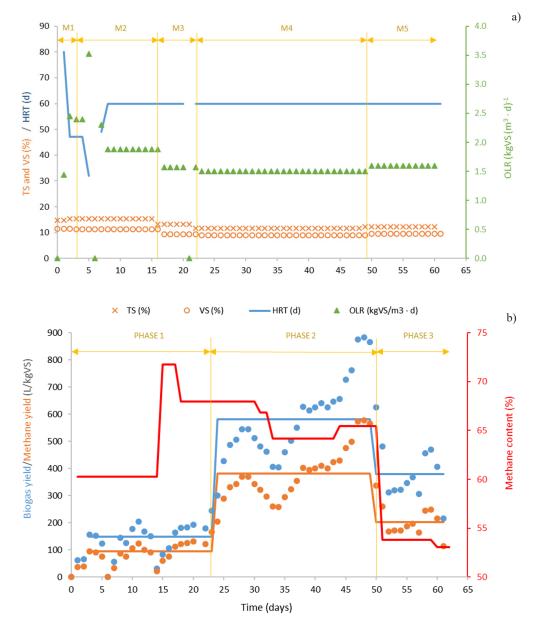


Figure 2. a) Operational conditions throughout the semi-continuous experiment. (x) %TS in the mixture fed; (\circ) %VS in the mixture fed; (-) hydraulic retention time (HRT), (\blacktriangle) organic loading rate (OLR); b) Daily biogas production (\bullet), Average biogas production for each phase (-), Daily methane production (\bullet), average methane production for each phase (-), methane content in biogas (-).

the loss of biomass and biogas. Overpressure inside the reactor was produced as a consequence of the obstruction of the gas outlet pipe to the flowmeter. As a measure to reduce foaming, OLR was reduced and HRT was increased. Furthermore, mixing intensity (mixing speed was increased and mixing interval was reduced) was also increased trying to improve liquid-gas mass transfer and release biogas contained in the digesting biomass. On day 8, HRT and OLR were fixed at 60 days and 1.6-1.9 kg VS m⁻³ d⁻¹. These changes led to a period of stability with high biogas production between days 24 and 49 (Phase 2). During this phase there were also some foaming incidents, but these were easily manageable and did not lead to significant operational problems. Operational conditions during this phase were constant at an HRT of 60 days and an OLR of 1.5 kg VS m⁻³ d⁻¹. Finally, during Phase 3 there was a slight decrease in biogas production, starting on day 50, despite operating the digester with the same conditions as in Phase 2. Mixture fed during Phase 3 used HD collected from the farm located in Tenerife (sample 3), which could have influenced biogas production.

Daily biogas and methane production are shown in Figure 2b, expressed as biogas and methane yield in L kg VS⁻¹. During Phase 1 average biogas and methane yield were 148.8±11.9 and 95.6±7.9 L kg VS⁻¹, respectively, increasing up to 582.0±29.5 L_{biogas} kg VS⁻¹ and 381.2±19.0 L_{CH4} kg VS⁻¹ in the second phase of operation. Finally, during Phase 3 biogas and methane yields decreased to 378.5±29.9 L_{biogas} kg VS⁻¹ and 203.0±16.0 L_{CH4} kg VS⁻¹. As already mentioned, Phase 1 was characterized by instability and operational problems, intrinsic to any startup process of an anaerobic digester. The reduction of the OLR and the increase in the HRT during this phase led to a stable period during Phase 2, with the highest biogas and methane production recorded in day 48 of operation, with a biogas and methane yield of 882.8 $L_{\rm biogas}~kg~VS^{-1}$ and 577.7 $L_{\rm CH4}~kg~VS^{-1}.$

Digester efficiency, expressed as methane production per volume unit of the digester and day ($L_{CH4} L_{dig}^{-1} d^{-1}$) (Table 3), was higher during Phase 2, with the highest reported value of $0.69\pm0.03 L_{CH4} L_{dig}^{-1} d^{-1}$ during week 7. Methane content was similar during Phase 1 and Phase 2, oscillating between 60 and 70%, decreasing afterwards in Phase 3 to approximately 53%. Hydrogen sulfide concentration was very high during Phase 2, reaching more than 2,000 ppm in week 5, decreasing afterwards in Phase 3 to 300-500 ppm.

Biodegradability is calculated based on the conversion of organic matter to biogas, and therefore, based on the reduction of organic matter in the digestate compared to the initial substrate fed to the digester. VS and COD reduction were used to determine biodegradability during this assay. Table 3 shows that, on average, VS reduction and COD reduction were higher than 50 and 65% throughout the experiment, respectively.

Table 4 shows all control parameters analyzed during the assay. TS and VS showed constant values during last weeks, indicating the stability of the reactor. IA, an indicator of volatile fatty acids (VFA), was high during the entire assay. VFA are intermediate compounds of the anaerobic digestion process. Their accumulation is an indicator of the decoupling of VFA production, by acidogenic and acetogenic bacteria, and VFA consumption, by methanogenic bacteria. Therefore, VFA accumulation showed an unstable process. In fact, the IA/PA ratio reached very high values during week 3 and 5.

As already mentioned, ammonia nitrogen can be inhibitory for the anaerobic digestion process. FA (NH_3) is considered the most toxic form of ammonia nitrogen as it is able to freely pass bacteria cell membrane (Rajagopal

Week	Y _{biogas} (L kgVS ⁻¹)	Y _{CH4} (L kgVS ⁻¹)	Digester eff. (L _{CH4} L _{dig} ⁻¹ d ⁻¹)	CH ₄ (%)	CO ₂ (%)	H ₂ S (ppm)	VS reduction (%)	COD reduction (%)
1	112.2±20.4	67.6±12.3	$0.17{\pm}0.04$	-	-	-	64.7	65.6
2	146.2±21.1	88.1±12.7	0.17 ± 0.02	60.3	39.7	729	74.5	-
3	128.6±21.6	86.9±14.1	0.15 ± 0.02	69.9	30.0	1215	47.3	63.3
4	305.3±47.8	207.4±32.5	$0.29{\pm}0.05$	-	-	-	29.2	-
5	493.6±18.7	329.1±15.1	$0.49{\pm}0.02$	67.9	31.8	2229	59.1	75.3
6	540.4±33.2	346.8±21.3	$0.52{\pm}0.03$	66.8	33.0	1856	53.7	-
7	704.5±34.2	458.7±23.2	$0.69{\pm}0.03$	-	-	-	52.2	78.6
8	543.9±95.4	321.7±68.6	$0.50{\pm}0.10$	53.8	46.1	571	56.6	-
9	391.7±33.3	209.7±13.6	0.33±0.03	53.5	46.5	305	60.4	68.8

Table 3. Biogas and methane yield (L kg VS⁻¹), digester efficiency ($L_{CH4}L_{digester}^{-1}$ day⁻¹), biogas composition (CH₄, CO₂, H₂S) and organic matter degradation (VS and COD reduction) during the semi-continuous assay (results reported in a weekly basis). Average ± standard error (calculated on a weekly basis from daily results).

Week	TS (%)	VS (%)	COD _t (g ₀₂ L ⁻¹)	COD_{s} ($g_{02} L^{-1}$)	PA (g _{CaCO3} L ⁻¹)	IA (g _{CaCO3} L ⁻¹)	IA/PA (-)	TKN (g L ⁻¹)	FA (mg L ⁻¹)	рН (-)
0	5.5	3.8	61.1	12.2	11.6	3.6	0.31	7.23	621	7.88
1	5.7	3.9	64.0	15.0	10.6	3.9	0.37	5.18	340	7.71
2	6.3	4.2	-	-	-	-	-	6.42	337	7.58
3	6.4	4.3	80.2	20.5	6.1	9.1	1.48	6.79	253	7.44
4	6.6	4.3	-	-	-	-	-	7.03	449	7.67
5	5.9	3.7	75.7	19.8	10.9	7.1	0.65	6.74	539	7.84
6	6.7	4.2	-	-	-	-	-	7.54	678	7.94
7	6.9	4.3	65.6	21.7	12.9	5.8	0.45	6.82	928	8.07
8	6.7	4.2	-	-	-	-	-	6.29	1278	8.21
9	6.6	3.8	50.7	18.5	14.950	5.083	0.34	6.07	1041	8.12

Table 4. Control parameters monitored during the semi-continuous assay.

TS= total solids; VS = volatile solids; COD_t = total chemical oxygen demand; COD_s = soluble oxygen demand; PA=partial alkalinity; IA= Intermediate alkalinity; TKN = Total Kjeldahl Nitrogen; FA = Free Ammonia.

et al., 2013). FA concentration, calculated according to Hansen *et al.* (1998), increased throughout the experiment up to concentrations higher than 1,000 mg L^{-1} at the end of the experimental period. The pH was stable during the experiment, oscillating between 7.44 and 8.21.

Energy availability

Methane yield during Phase 2 of the semi-continuous assay was used to calculate the available energy for the farm use. Methane yield considered for the calculation was therefore 381.2 L_{CH4} kg VS⁻¹. The amount of HD produced daily by the farm is 1.4 ton d⁻¹ with an average TS and VS content of 31.4% and 22.8%, respectively (only sample 1 and 2 were considered for calculating the average composition, since these samples were collected from the farm being studied). In order to obtain a mixture with a C/N ratio higher than 10 and with a TS content lower than 20%, the amount of GCW (average composition of 8.6 %TS and 7.6 %VS) to be added is 3.3 m³ d⁻¹, leading to a mixture with a TS and

VS content of 15.5 and 12.9 %, respectively, and with a C/N ratio of 10.3.

Daily methane production treating 4.8 m³ d⁻¹ of the mixture is estimated to be 233.5 m³ d⁻¹, leading to a daily electricity and heat production of 697.6 kWh d⁻¹ and 1,395.1 kWh d⁻¹. CHP unit power would be 30 kWe, if biogas is to be used continuously throughout the day. On the other hand, the amount of digestate to be commercialized would be around 1,600 m³ year⁻¹. Figure 3 shows a scheme of the valorization path of HD and GCW through anaerobic co-digestion.

The economic feasibility study was performed considering several assumptions that would influence the results. All these assumptions can be found in the M&M section. With these assumptions, results showed that such an investment would only be profitable if investment costs are close to $5,000 \notin kWe^{-1}$ (150,000 \notin for the full plant), receiving a subsidy of 67,500 \notin and leading to a NPV of 56,355.01 \notin and an IRR of 13.92%. In contrast, if the highest investment cost is applied (9,000 $\notin kWe^{-1} = 270,000 \notin$ for the

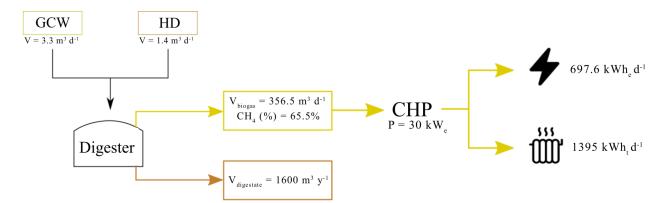


Figure 3. Valorization scheme of hen droppings (HD) and goat cheese whey (GCW) through anaerobic co-digestion, energy and digestate availability. CHP = combined heat and power unit.

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full plant), NPV of the investment would be negative (-5,909.14 \in) and IRR 5.48%, despite receiving a subsidy of 121,500 \in (45% of the investment cost) (see Table S2 [suppl.]).

Discussion

Substrates characterization

Composition of HD hinders its anaerobic digestion. On the one hand, the low moisture content prevents its use as substrate for wet anaerobic digestion, which requires a TS content below 20%. This fact would lead to the use of dry anaerobic digestion unless the substrate is diluted with water (with the consequent cost for the process) or co-digested with other liquid substrate which increases the moisture content of the resulting mixture. On the other hand, its C/N ratio is lower than the C/N ratio recommended for anaerobic digestion, which is between 10 and 30 (Schattauer & Weiland, 2004). The low C/N ratio is an indicator of possible problems during the digestion process due to ammonia inhibition (Rajagopal et al., 2013) and shows the need for looking for possible solutions prior to the digestion of HD as sole substrate, such as co-digestion with substrates with a higher C/N ratio.

GCW showed a high moisture content, like other typical wheys analyzed in literature (Carvalho *et al.*, 2013), with a remarkable content of organic matter in its dry matter, which indicates a high biodegradability. Its low pH highlights its acid character, which could lead to problems during the anaerobic digestion process depending on the OLR used, since optimum pH for the anaerobic digestion process is close to neutral. The high biological activity of GCW indicates a constant process of decay which according to Powell *et al.* (2013) does not influence its biogas potential. Finally, its C/N ratio is high and ideal for the anaerobic digestion process.

Comparing whey composition with other studies it has to be highlighted the high dry matter content of the whey used in this research. For instance, Antonelli *et al.* (2016) used a whey with TS and VS content of 5.9% and 5.3%, respectively, whereas Carlini *et al.* (2015) studied biogas production from a whey containing 5.9%TS and 5.8%VS and Hublin *et al.* (2012) from whey containing 4.69%TS and 4.26%VS. Probably, this difference in TS content is consequence of the whey origin (goat milk) and the traditional way of making the cheese in the Canary Islands, which is normally less efficient than an industrial process. In fact, Jasko *et al.* (2011), authors that used CW obtained from a homemade cheese maker, obtained a whey with 7.2% TS and 6.6% VS, values closer to the whey used in this study. On the other hand, it is also remarkable that C/N ratio of GCW in this study is much higher than C/N ratios of wheys used in other anaerobic digestion tests, which were 5.8 for Carlini *et al.* (2015) and 8.7 for Hublin *et al.* (2012). In these cases, whey cannot be used as a co-substrate to increase C/N ratio.

The comparison of the composition of both substrates showed an excellent complementarity for the anaerobic digestion process. The whey provides a high amount of water to the mixture, along with significant amounts of biodegradable organic matter. This high-water content is enough to dilute dry matter concentration of HD to a concentration close to 15-20%, which would be enough for treating the mixture by wet anaerobic digestion, which is simpler and normally has a higher kinetics than dry anaerobic digestion thanks to a faster hydrolysis reaction (Veeken *et al.*, 2000; Kothari *et al.*, 2014). On the other hand, the use of GCW as co-substrate increases the C/N ratio of the mixture, being enough for achieving C/N ratios close to the optimum values for the anaerobic digestion process.

Biochemical methane potential (BMP) assays

As shown in Fig. 1, methane production was delayed at the beginning of the assay. This delay was probably consequence of two different factors: the reactors' headspaces were not flushed, and the inoculum was not previously adapted to the substrates used. Afterwards, methane production continued without any problem.

Methane production of HD was similar to other studies. Niu *et al.* (2013) showed methane yields between 254.2 and 177 L_{CH4} kg VS⁻¹ before the inhibition of the process occurred. In other study, Molaey *et al.* (2018) reached a methane yield of 260 L_{CH4} kg VS⁻¹ supplementing chicken manure with trace elements in order to mitigate ammonia inhibition. It must be noted that both authors studied the process in continuous mode and under high ammonia concentrations that led to inhibition of the process. In this study, batch assays showed slightly higher methane yields (282.1 ± 3.98 L_{CH4} kg VS⁻¹) with inoculum which was not adapted to the substrate used.

On the other hand, GCW biogas production was high and similar to other studies that used cow CW, indicating that CW, regardless of its origin, is a great substrate or co-substrate for biogas production. Methane yield obtained in this study (691.9 \pm 42.1 L_{CH4} kgVS⁻¹) was similar to results obtained by Yan *et al.* (1988) in an Upflow Anaerobic Sludge Blanket (UASB) in continuous mode, who showed the highest methane yield for CW to be 533 L_{CH4} kg COD⁻¹, equivalent to approximately 756.9 L_{CH4} kg VS⁻¹, according to characterization of CW showed by authors. Escalante

et al. (2018) showed methane yields ranging from 510 to 600 L_{CH4} kgVS⁻¹ with four different CWs obtained from four dairies in Colombia. Contrarily, Antonelli *et al.* (2016) showed low methane yield digesting whey in batch mode with 170.1 and 104.3 L_{CH4} kg VS⁻¹ at 32 °C and 26 °C, respectively. Probably, low yields obtained by Antonelli *et al.* (2016) were consequence of an acidification process occurring during the digestion, consequence of the high whey/inoculum ratio used for the study.

Results showed the high potential of the GCW as a substrate or co-substrate to produce biogas. Addition of GCW improved substantially the methane yield of HD digested alone (p<0.05). Therefore, it can be a valuable co-substrate for high concentrated wastes with low C/N ratio. In fact, several authors explored this possibility in the past (Kavacik & Tapaloglu, 2010; Comino *et al.*, 2012; Hublin *et al.*, 2012; Carlini *et al.*, 2015), however, few of them studied the process in batch mode.

Hublin et al. (2012) showed that in batch mode the addition of whey to cow manure in high ratios (>5% v/v) required the addition of alkalinity (in form of NaHCO₂) in order to avoid inhibition due to acidification of the reactors. Furthermore, Carlini et al. (2015) showed also that a high proportion of cheese whey in the mixture with poultry manure (3:1 cheese whey: poultry manure-v/v) led to acidification and disruption of the process. On the other hand, if no acidification occurred, the highest the proportion of cheese whey in the mixture with poultry manure, the highest the methane yield, increasing 65.2% the methane yield when CW:poultry manure ratio was increased from 1:3 to 1:1. Acidification was not observed in this study with a mixing ratio of 3:1 (GCW:HD), highlighting the importance of using appropriate inoculum/substrate ratio in batch assays to avoid inhibition. Furthermore, results of other studies showed the importance of monitoring pH variations in continuous anaerobic reactors when digesting or codigesting whey due to its acidic nature.

In any case, all studies showed the potential of co-digesting CW with animal manure, regardless of its origin (cow or chicken) and their mixing ratios, as long as acidification does not occur. Due to the increase in methane yield when co-digesting HD and GCW, compared to the mono-digestion of HD, semicontinuous assay was performed with the mixture of HD and GCW in order to explore biogas yield and further operational issues in a laboratory setup similar to full-scale plants.

Semi-continuous assay

The characterization of the mixtures used during the semi-continuous assay showed a high CODs fraction,

being greater than 50 g O₂ L⁻¹ in all mixtures, representing at least between 16.3 and 30.8% of the CODt. This high soluble fraction indicated a high biodegradability and, probably, a fast kinetics of degradation, which is beneficial for the anaerobic digestion process. On the other hand, it can lead to sudden changes in pH, which can be detrimental to the digestion process. Moreover, the mixture itself had a low pH. Finally, TKN and TAN content were high. As already said, high N can lead to inhibition due to ammonia accumulation inside the digester. C/N ratio was increased from 7.18 (lowest C/N ratio of HD) to more than 10, thanks to the mixture of HD and GCW. Mixtures also showed significant variability in their composition, as a consequence of keeping C/N ratios higher than 10. These changes, otherwise, would be common in an agro-industrial biogas plant, where the composition of substrates varies with the season, process conditions (e.g. in cheese production), animal feeding, etc.

During the semi-continuous assay foam production was constant, with periods of high production that led to severe operational problems: biogas loss and digester overflow. Foam production is common in anaerobic digesters (Kougias *et al.*, 2014). This problem is usually related to a high organic load, instability of the digestion process, presence of filamentous microorganisms, type of substrate fed into the digester, inadequate mixing and temperature changes (Evans *et al.*, 2011; Kougias *et al.*, 2013a).

Kougias et al. (2014) investigated foaming incidents in a full-scale biogas plant concluding that they were consequence of the substrates fed into the digester and the mixing pattern. In particular, foaming incidents were attributed to the use of acid substrates (whey) and substrates with a high protein content (chicken manure), being similar substrates as the used in this study (GCW and HD). Therefore, foaming problems in the digester were probably consequence of the codigestion of GCW and HD. A decrease of the OLR and an increase of the HRT was shown to be effective in reducing foaming production, although it was almost permanent throughout the study. Therefore, when scaling up the process, special measures should be considered in order to control foaming phenomenon inside the digester, such as constant foam monitoring and antifoamers dosing (Kougias et al., 2013b).

Biogas and methane yields during the assay were high compared to anaerobic reactors working only with animal manures, where methane yields are commonly between 200 and 300 L_{CH4} kg VS⁻¹ (Biogas3, 2014). During the phase with higher biogas production (Phase 2), biogas and methane yields were 582.0±29.5 L_{biogas} kg VS⁻¹ and 381.2±19.0 L_{CH4} kg VS⁻¹, with a maximum production of 704.5±34.2 L_{biogas} kg VS⁻¹ and 458.7±

23.2 L_{CH4} kg VS⁻¹ during week 7. Biogas production decreased suddenly during Phase 3 (week 8 and 9), when HD used for the mixture was collected in the second farm, located in Arico (Tenerife). Probably, the change of substrate caused an unidentified disruption in the process, which led to a lower biogas yield and a decrease in the methane content in biogas.

Other studies have shown similar biogas yields for the co-digestion of CW and animal manure. Comino *et al.* (2012) showed a methane yield between 195 and 386 L_{CH4} kg VS⁻¹ when co-digesting a mixture of cow manure and CW with varying content of each substrate at 42 days of HRT. The highest biogas production was obtained for the mixture containing the highest fraction of CW (65% CW and 35% cow manure (v/v)), like the mixture used throughout the semi-continuous assay in this study and with similar results. It must be noted that Comino *et al.* (2012) did not observe any foam in the digester: the gradual increase in the proportion of whey in the mixture and/or the substitution of chicken manure for cow manure could be the reasons.

Table 3 shows biodegradability of the mixture fed into the digester. At the end of the process, when the digester was closer to equilibrium, organic matter removal was higher than 50% (in terms of VS removal) and 65% (in terms of CODt removal). Large HRT favor organic matter removal, therefore, these values are expected to decrease in an anaerobic reactor operated at shorter HRT, which is the desired situation in full-scale plants in order to obtain a higher energy efficiency of the reactor. Removal of COD was like that observed by Comino et al. (2012) when co-digesting cow manure and CW in similar fractions. In a CSTR the digester is not in equilibrium for certain conditions until it is operated $1.5-2 \times HRT$ without any change. Results on biodegradability are subject to equilibrium state within the digester. Since in this study the experiment lasted 60 days (1×HRT), the results shown here should be taken with caution.

During the experiment IA was very high, indicating high accumulation of VFA. Furthermore, IA/ PA ratio was always above 0.3, which is considered to be the threshold for an unstable digestion process (Ripley *et al.*, 1986). The instability of the process, and more precisely, the accumulation of VFA favor foam production (Kougias *et al.*, 2013a). Despite the imbalance of the digestion process, biogas production and methane content in biogas was high during most of the assay, with a decrease during the two last weeks of operation.

FA concentration throughout the assay increased since the beginning of the experiment, reaching values higher than 1,000 mg L⁻¹. There is no consensus regarding the threshold above which FA concentra-

tion is toxic for anaerobic microorganisms, moreover, microorganisms have the ability of adapting to increasing FA concentrations (Ramos-Suárez *et al.*, 2014). Some authors have observed signs of inhibition even for FA concentrations above 100 mg L⁻¹ (Parkin & Owen, 1986), although it is normal to observe the toxic concentration threshold above 700 mg L⁻¹ for animal manures (Rajagopal *et al.*, 2013). Therefore, it is possible that the increasing accumulation of FA in this study caused the decrease in biogas yield observed during the last two weeks of operation.

Overall, the increase in the energy yield when mixing HD and GCW could make profitable, through energy self-consumption and selling, the investment in such a processing plant. Furthermore, GCW could be properly treated by anaerobic digestion.

Results found during this study are directly applicable to the farm under study. Any extrapolation to other farms in the Canary Islands should be done with caution due to the methodology used, which was focused only on this laying hen farm in Fuerteventura.

Energy availability

The laying hen farm being studied would have available a large amount of electricity, which would be able to cover all the electricity demand by the farm. Even peak demand would be cover, considering that the CHP unit which could be installed (30 kWe) would be higher than the electric power currently installed in the farm (27.7 kWe). Electricity production from biogas which exceeds electricity demand by the farm could be sold to the electricity network. According to average cost of electricity for the farm, savings thanks to electricity self-consumption could reach 8,176 € year⁻¹, whereas incomes due to the sale of electricity to the electricity network could be $11,724.81 \in \text{year}^1$, summing up 19,900.81 € year⁻¹. On the other hand, heat energy would also be available. Heat energy is currently not necessary on the laying hen farm, but new processes could be implemented in order to use this available energy. In summary, the co-digestion of HD and GCW would create a new way of waste valorization. On the other hand, cheese manufacturers would save in GCW management.

The application of small-scale biogas plants is often difficult due to economics of scale and low energy yield from manure (Deublein & Steinhauser, 2008; Klavon *et al.*, 2013; Scheftelowitz & Thrän, 2016). Therefore, the use of a co-substrate could improve the economics of such investment due to higher energy production and higher scale of the biogas plant. This model needs the cooperation between cheese manufacturers and the laying hen farm to provide successful exploitation results, whereas the economic viability of such a model would depend on the biogas technology used, subsidies for the application of anaerobic digestion for waste treatment and self-energy consumption of the farm, since in Spain feed-in tariffs for renewable energy were suspended in 2012.

A rough economic feasibility study has been made in order to estimate if the investment in such a treatment system would make sense for the laying hen farm. Results showed that the investment in such a facility is economically feasible only if the investment costs are low (around 5,000 \in kWe⁻¹). If the investment costs rise to the high values of the range (around 9,000 \in kWe⁻¹), which is according to other authors more common for small scale biogas projects (Scheftelowitz & Thrän, 2016), the investment would not be feasible even with a 45% subsidy of the investment.

Results showed that even with an increase in energy production due to the co-digestion of HD with GCW the investment should be reduced as much as possible in order to make feasible such a processing plant. Other possible sources of income, such as waste management fees or digestate selling, were not considered in this evaluation and could improve the economics of the processing plant. Digestate may have a higher acceptance by local farmers thanks to a reduction of odors, pathogens, seeds and a higher nutrient availability for crops (Al Seadi & Lukehurst, 2012) compared to raw manure.

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