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1 **Repeated application of anaerobic digestate, undigested cattle slurry and inorganic**
2 **fertiliser N: impacts on pasture yield and quality**

3

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9

10 *Running title:* Impact of organic & inorganic fertiliser additions on pasture quality

1 **Abstract**

2 The growth of on-farm anaerobic digestion (AD) has generated significant quantities of
3 digestate for use as a soil amendment. However, relative to other organic and inorganic
4 fertilisers, only limited field trial data exists on the effect of repeated applications of
5 digestate to temperate grasslands. Here, we compare yields and grass quality (protein and
6 digestibility) from a field trial of a mixed pasture ley (ryegrass and clover), following the
7 application of five different fertiliser types (liquid digestate generated from anaerobically
8 digested slurry, dry fibre digestate, undigested slurry, ammonium nitrate and an NPK
9 compound fertiliser) in comparison to a no-fertiliser control. Application rates were
10 normalised in terms of nitrogen (N) and were added as a split dose, with 100 kg N ha⁻¹
11 added prior to the first harvest and an additional 50 kg N ha⁻¹ supplied after the first
12 harvest, every year for three years. Overall, our results showed that applying both forms of
13 digestate or undigested slurry gave grass yields that matched those obtained with one
14 compound inorganic fertiliser, and better than those from a straight N inorganic fertiliser.
15 No differences were found with regards to digestibility or sward N-content (hence
16 calculated protein) between any treatments. Although the trial was conducted at only one
17 site, the results indicate that inorganic fertilisers can potentially be replaced by digestate
18 without compromising grassland productivity.

19

20 *Keywords:* Biogas, Feed value, Greenhouse gas emissions, Livestock

21

1 **1. Introduction**

2 As global demand for food continues to increase, so too will agricultural demand for
3 nutrients. Satisfying this demand through reliance on inorganic (synthetic) fertiliser is
4 expensive. It also increases greenhouse gas (GHG) emissions due to the energy-intensive
5 production process (Ecoinvent, 2007) and emissions from soils, post-application. These
6 economic and environmental concerns, coupled with increased demand for low-carbon
7 energy, mean that there is increasing interest in anaerobic digestion (AD) as an alternative
8 source of nutrients (Demirer & Chen, 2005). AD is the decomposition of organic resources in
9 the absence of oxygen to produce biogas (typically 50-70% methane) and a digestate
10 fertiliser (Masse et al., 2011). The biogas can be burnt to generate renewable energy and
11 heat, and the digestate is an organic fertiliser which can be applied to land. The digestate
12 can be separated into liquid and dry fibre portions, and although it varies with the
13 separation process used, the dry fibre retains the greatest amount of P and has a similar
14 texture to compost (dry matter (DM) content of ~20%); whilst typical DM for the liquid
15 digestate will be 4-6%, and is associated with low P and high N contents (Moeller et al.,
16 2010).

17 Inorganic fertilisers are often considered to represent a more effective and
18 controllable source of plant nutrients than organic fertilisers; however, the latter enhance
19 soil organic matter which improves soil structure, porosity and drainage (Choudhary et al.,
20 1996), especially so dry fibre digestate, which has higher levels of organic matter. Further,
21 as the release of nitrogen from organic sources is sustained over a longer period, there is
22 often a residual effect from the mineralisation of nitrogen following repeated applications
23 of organic fertilisers (Gutser et al., 2005). Compared to undigested animal manures,
24 digestate has potentially greater plant-available nitrogen due to a greater proportion being
25 in the form of ammonium (NH_4^+) (Möller & Müller, 2012). Nevertheless, surprisingly few
26 field studies have sought to ascertain the agronomic benefits of digestate application;
27 particularly so on temperate grasslands that sustain ruminant livestock farms (Möller et al.,
28 2008; Walsh et al., 2012a). This is surprising given that AD systems are frequently found on
29 such farms due to the availability of manures which make suitable feedstock for AD
30 (Demirer & Chen, 2005). Furthermore, the impact of digestate application on forage quality
31 (e.g. protein content and digestibility) has yet been studied.

1 The aim of this study was to determine the effects of the repeated application of
2 different fertilisers, including AD digestate, on a mixed pasture ley over three growing
3 seasons. The key indicators used to evaluate treatment performance were dry matter yield,
4 pasture composition, forage protein and digestibility.

6 **2. Materials and methods**

7 *2.1 Experimental design*

8 The experimental field site was located on freely draining agricultural grassland
9 located at Bangor University's Henfaes Research Centre, North Wales (53°14'05''N,
10 4°00'50''W). The sward contained a newly sown mixture of perennial ryegrass (*Lolium*
11 *perenne* L.) and white clover (*Trifolium repens*, L.) and was previously subject to sheep
12 grazing (ca. 15 ewes ha⁻¹). The soil has a clay-loam texture and is classified as a Eutric
13 Cambisol (of the 'Denbigh' series) and is derived from mixed glacial till. The trial was
14 conducted over a three year period (2011-13).

15 Five different fertiliser treatments were applied to 2 × 2 m plots (*n* = 4 plots per
16 treatment), organised in a randomised design and separated by a 1 m wide buffer. These
17 included: a no fertiliser control (C); undigested cattle slurry (US); the liquid fraction of
18 anaerobically digested cow slurry (liquid digestate, LD); the dry fibre fraction of
19 anaerobically digested cow slurry (dry fibre digestate, DFD); inorganic N 34.5% fertiliser (N;
20 ammonium nitrate) and an inorganic NPK 21-8-11 (NPK) compound fertiliser (N in the form
21 of both ammonium and nitrate; Yara (2017)). The US was collected fresh from an organic
22 dairy farm (from lactating cows) and both digestates were collected from an AD unit on the
23 same farm. The AD unit is a 1000 m³ mesophilic (38 °C) gas mixing system, continually
24 stirred digester with a retention time of 25 days and fed with cow slurry only. The system
25 separates the digestate mechanically through a continuous belt press after leaving the
26 digester, which enabled the liquid and solid fraction could be collected. Samples were
27 refrigerated then analysed within 24 h of collection.

28 In all, six above-ground vegetation harvests were performed on the plots, with two
29 harvests taken per year (May-June and August-September) to a height of 4 cm above the
30 soil. Only the plant biomass within the central 1 m² of the plots was quantitatively

1 evaluated, to avoid potential edge effects. To represent farmer practice, an initial
2 application of 100 kg N ha⁻¹ of each fertiliser type was surface-applied in mid-April, and the
3 first harvest each year was undertaken six weeks later. A second fertiliser addition of 50 kg
4 N ha⁻¹ was then applied one week post-harvest (after some grass regrowth) and the final
5 harvest taken six weeks thereafter. The application rate for each was normalised for
6 nitrogen, based on total nitrogen content.

7

8 *2.2 Soil, fertiliser and plant analysis*

9 Soil samples were taken to a depth of 15 cm from each plot at the beginning of the
10 experiment. Soil and organic fertiliser were extracted with 1 M KCl (1:5 (w/v)), shaken (250
11 rev min⁻¹, 1 h, 20 °C), centrifuged (4000 g, 15 min) and the supernatant filtered (Whatman
12 no. 42) (Jones and Willett, 2006). Major cations (K⁺, Na⁺ and Ca²⁺) were analysed using a
13 model 410 flame photometer (Sherwood Scientific, Cambridge, UK), whilst NO₃⁻, NH₄⁺ and P
14 were determined colorimetrically (Synergy[®] Microplate Reader; BioTek US, Winooski, VT)
15 using the methods of Mulvaney (1996), Miranda et al. (2001) and Murphy and Riley (1962),
16 respectively. Total organic carbon and nitrogen were measured using a CHN2000 elemental
17 analyser (Leco Corp., St Joseph, MI), and dissolved organic carbon (DOC) and dissolved
18 organic N (DON) were determined using a TCN-V analyser (Shimadzu Corp., Kyoto, Japan).
19 Samples were oven dried at 105 °C for 24 h to determine gravimetric water content.
20 Electrical conductivity (EC) and soil pH were determined using standard electrodes in 1:5
21 (w/v) distilled water extracts.

22 Forage digestibility was analysed from all treatments for the final two harvests (year
23 three only). With the exception of the first harvest, the harvested material was manually
24 separated to determine the proportion of grass and clover in the sward. All harvested plant
25 material was weighed fresh, and then a 300 g subsample was removed, dried at 85 °C for 48
26 h, and reweighed. Crop nutrient analysis was undertaken using traditional wet chemistry
27 (Givens et al., 2000) for both harvests in year three of the trial to determine total nitrogen
28 content of the shoots. Digestibility was calculated using the modified acid-detergent fibre
29 content of each sample (Givens et al., 2000).

30

1 *2.3 Statistical analyses*

2 Statistical analysis was performed using SPSS v.18 (IBM UK Ltd., Hampshire, UK). For
3 analysis of crop yield data, total yield from all harvests were used and subject to a one-way
4 ANOVA to determine differences within each sub-group, with treatment as the factor. The
5 same analysis was used for nitrogen and digestibility tests. Post-hoc tests were carried out
6 on all ANOVAs using Tukey HSD test at the level $p < 0.05$.

7

8 **3. Results**

9 *3.1 Soil and fertiliser characterization*

10 Table 1 reports the physico-chemical properties of the soil at the onset of the trial,
11 and the mean of the three organic fertilisers used over the duration of the field trial. As both
12 the feedstock used (cattle slurry) and the management of the digester remained constant
13 throughout the trial period, there was comparatively little variability in the components of
14 the digestate generated.

15

16 (Table 1 here)

17

18 *3.2 Forage yields*

19 The yield of grass obtained was slightly lower than typical values for a new pasture ley,
20 although not so by year three of the study (AHDB, 2017). Yields were considerably greater in
21 year three for all treatments and the control. The cumulative forage yield across all six
22 harvests showed that plots applied liquid digestate (LD) had the greatest yield; indeed, yield
23 was significantly greater ($p < 0.05$) compared to the straight inorganic N treatment, but not
24 significantly different ($p > 0.05$) from pasture applied undigested slurry (US), digestate fibre
25 (DFD) or NPK fertiliser. There was no significant difference between the two inorganic
26 fertiliser treatments ($p > 0.05$).

27

28 (Figure 1 here)

29

1 *3.3 Sward composition*

2 Cumulatively over the three years' harvests, the unamended control had the greatest
3 proportion of clover (of total yield), and was significantly greater ($p < 0.05$) from all other
4 treatments (Table 2). Of the treatments, pasture applied US produced the greatest clover
5 yield; although this difference was only significant ($p < 0.05$) to swards amended with
6 inorganic N and not to any of the other organic amendments. There were no differences in
7 clover percentage between pasture applied LD and DFD and that applied the two inorganic
8 fertilisers ($p > 0.05$).

9

10 (Table 2 here)

11

12 *3.4 Sward nitrogen content*

13 Within ryegrass, all treatments had greater levels of nitrogen ($p < 0.05$) than control,
14 but there were no differences between any of the other fertiliser treatments ($p > 0.05$); all
15 being 28.8-35.2 g kg⁻¹. With respect to clover, the DFD treatment possessed the greatest
16 amount of nitrogen of all treatments (mean of 48.2 g kg⁻¹), being significantly different from
17 the inorganic N and NPK treatments, which possessed the lowest N levels (40.6-42.4; $p <$
18 0.05). Again, there was no statistical difference ($p > 0.05$) between the other treatments.

19

20 *3.5 Forage digestibility*

21 Grass applied LD had the lowest digestibility of all treatments at 60%, while all other
22 treatments reported a value of 62-66%; however, none of these differences were
23 statistically significant ($p > 0.05$).

24

25 **4. Discussion**

26 As well as being a source of renewable energy, anaerobic digestion is increasingly seen
27 as a more sustainable way of recycling nutrients from wastes and by-products to land.
28 However, surprisingly few studies have reported on the agronomic impacts of digestate
29 application. The results presented here show that, cumulatively, application of organic

1 fertilisers derived from anaerobic digestion gave similar yields of grass to when an NPK
2 compound fertiliser were repeatedly applied over a three year period, and statistically
3 greater yield than when straight N inorganic fertiliser was applied. Only a limited number of
4 studies have compared the agronomic value of slurry-derived digestate relative to
5 undigested slurry and inorganic fertiliser, and direct comparison of the results between
6 studies is often difficult due to variability between feedstocks, the AD process (hence
7 digestate properties), crops, and the rates and methods of digestate application (WRAP,
8 2016). Further, many such studies are based on pot trials set under controlled conditions
9 (Möller and Müller, 2012) and only one growing season. In field trials over a longer
10 harvesting period, the extra rooting volume of crops compared to pots may lead to the
11 acquisition of mineralised organic nitrogen from manures by crops (Morris & Lathwell, 2004;
12 Möller et al., 2008; Svensson et al., 2004). In this study, forage yield values increased with
13 time, probably due to the third year being a wetter year than average for the area (data not
14 shown) and hence facilitating growth. Further, there was no statistically significant
15 difference from the application of undigested or digested slurry on forage yield over a three
16 year period; which is in contrast with other studies mentioned previously. The variability in
17 factors that govern growth with time reiterates the value in conducting trials over multiple
18 years.

19 In comparison to straight ammonium nitrate inorganic fertiliser, organic fertilisers
20 provide additional nutrients in the form of phosphorus and potassium; which may explain
21 the lower yields from pasture applied the former in this trial (Figure 1). In the longer term,
22 digestate application, relative to inorganic fertiliser may also increase soil organic matter
23 and hence improve nutrient retention and overall soil quality, as well as provide residual
24 effects due to the slower release of nutrients applied (Gutser et al., 2005). As expected, the
25 nutrient profile of the separated (liquid and dry fibre) digestate components differed.
26 Although it comes at some cost to the operator, separation of the digestate into the
27 different fractions can therefore facilitate better targeting of nutrients according to crop
28 and soil demands, in addition to reducing haulage and storage costs of dealing with all
29 digestate as liquid.

30 Bougnom et al. (2012) reported greater percentage of legumes in soil treated with
31 undigested manure rather than digestate, which corroborates our results (Table 2). Hakala

1 et al. (2012) also reported that there was greater prevalence of clover plants (albeit
2 insignificantly so) where organic fertiliser had been applied compared to inorganic fertiliser
3 at the end of a three year field trial. The application of nitrogen is frequently reported to
4 suppress clover growth, thereby reducing the beneficial supply of free nitrogen through N-
5 fixation (Hakala et al., 2012; Nesheim et al., 1990). At the end of this study, control plots had
6 the greatest percentage of clover, followed by pasture applied US, which were both
7 statistically greater than all other fertilised treatments (Table 2). Thus, although digestate is
8 an organic fertiliser, it may restrict clover growth over time in a similar way to inorganic
9 fertiliser, possible due to the higher amount of plant-available nitrogen in digestate fertiliser
10 compared to US (Walsh et al., 2012b). This may need further consideration given the dietary
11 benefits to livestock of the presence of clover in grass swards and silage (Fraser et al., 2004).

12 Although converting foliar nitrogen content to leaf protein by multiplying by 6.25
13 tends to overestimate the true protein content of feedstocks, it is widely accepted as
14 industry standard (Sriperm et al., 2011). After three years of fertiliser application, little
15 difference in N/protein content was seen between all five fertiliser treatments. This is of
16 note, as protein content is an important parameter of feed value, and the results indicate
17 that other factors (e.g. fertiliser application rate, species of forage crop) have a greater
18 bearing than fertiliser type on forage protein status.

19 Along with protein content, the feed value of grass is largely governed by the
20 digestibility of the forage. In this study, no difference in relation to digestibility was reported
21 between treatments. Digestibility of plant tissue typically increases with N fertilisation
22 (Johnson et al., 2001; Messman et al., 1992; Prine & Burton, 1956). The fact that all harvest
23 were taken within six weeks of fertiliser application, before any rapid decrease in
24 digestibility, may explain the lack of treatment effect. It would be beneficial to have a longer
25 harvesting time to determine if differences in digestibility would occur after an additional
26 two-three weeks' growth.

27

28 **5. Conclusion**

29 Although this research was conducted on one crop and soil type and at one
30 geographical location, it demonstrates the potential value of digestate as a nutrient source

1 for pasture systems. Further, the study implies that application of digestate leads to a
2 similar response in pasture yield as when a compound inorganic fertiliser is applied, and
3 better than straight N organic fertiliser. Any agronomic benefits of replacing inorganic
4 fertiliser use with digestate should be viewed alongside the long-term wider environmental
5 benefits (e.g. in reducing GHG or loss of N to freshwater) and the potential value of
6 digestate in raising soil organic matter levels.

7

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22

1 **Figure 1:** Annual dry matter (DM) grass yield over the three-year field trial, following the
2 application of different fertiliser types: no-fertiliser control (C), undigested slurry (US), liquid
3 digestate (LD), dry fibre digestate (DFD), inorganic fertiliser (N) and compound inorganic
4 NPK fertiliser (NPK). Values represent means \pm SEM ($n = 4$).
5

1 **Table 1:** Mean physico-chemical properties of the soil and of the organic fertilisers
 2 (undigested slurry (US), liquid digestate (LD) and dry fibre digestate (DFD)) used over the
 3 three-year field trial. Values represent means \pm SEM ($n = 9$) and are expressed in terms of
 4 dry weight, where applicable.

5

	Soil	Organic fertiliser		
		US	LD	DFD
pH	5.45 \pm 0.02	7.07 \pm 0.24	8.44 \pm 0.12	8.89 \pm 0.13
EC (mS cm ⁻¹)	44.4 \pm 5.4	5.68 \pm 1.68	6.57 \pm 2.82	8.12 \pm 6.05
Dry matter (%)	78.3 \pm 0.2	14.4 \pm 1.6	4.9 \pm 1.6	24.3 \pm 1.4
Total C (mg g ⁻¹)	ND	393 \pm 8	214 \pm 6	289 \pm 8
Total N (mg g ⁻¹)	ND	30.2 \pm 5.4	66.4 \pm 4.9	38.4 \pm 6.4
C-to-N ratio	–	27.7	10.2	34.4
DOC (mg g ⁻¹)	0.1 \pm 0.0	58.2 \pm 13.0	47.2 \pm 14.9	29.2 \pm 5.7
DON (mg g ⁻¹)	ND	13.2 \pm 0.9	27.3 \pm 4.9	10.1 \pm 1.8
NO ₃ ⁻ – N (mg g ⁻¹)	<0.1	0.44 \pm 0.07	0.35 \pm 0.08	4.24 \pm 4.01
NH ₄ ⁺ – N (mg g ⁻¹)	<0.1	15.39 \pm 4.68	38.55 \pm 5.14	23.58 \pm 0.23
P (mg g ⁻¹)	0.09 \pm 0.01	11.70 \pm 2.29	3.69 \pm 1.42	6.16 \pm 1.08
K (mg g ⁻¹)	0.03 \pm 0.01	12.53 \pm 3.33	13.41 \pm 1.95	12.73 \pm 3.85
Ca (mg g ⁻¹)	0.04 \pm 0.01	10.50 \pm 3.90	8.30 \pm 5.60	2.30 \pm 0.60
Na (mg g ⁻¹)	0.07 \pm 0.01	2.86 \pm 0.85	8.12 \pm 3.06	7.80 \pm 1.40

6 ND: not determined

7

1 **Table 2:** Percentage of clover in the yield of forage harvested over the three-year field trial,
2 following the application of different fertiliser types (Control received no fertiliser). Letters
3 denote significant differences between treatments ($p < 0.05$).

4

Treatment	Clover (% of DM yield)
Control	34.9 ^a
Undigested slurry	29.4 ^b
Liquid digestate	22.1 ^{b c}
Dry fibre digestate	22.1 ^{b c}
N inorganic fertiliser	22.2 ^c
NPK compound inorganic fertiliser	21.6 ^c

5