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A proposal for enhanced EU herbage VCU and DUS testing procedures

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Abstract

Herbage production is regarded as having environment-friendly credentials. However, as the ruminant production it supports is facing major challenges on sustainability, environmental footprint and human health concerns, EU herbage cultivar testing must contribute to the solutions. Before new cultivars can be sold in a member state (MS) and gain EU-wide marketing, they must pass official tests to prove they are both novel (distinct, uniform and stable, DUS) with improved value for cultivation and use (VCU). Herbage species present specific challenges, as their allogamy imposes a wide within-cultivar variation that adds complexity to DUS tests and their "value" is only realized in ruminant produce. Current VCU systems measure production, chemical composition and disease/stress tolerances, often on large numbers of candidate cultivars, but prohibitive labour costs and logistics mean that animal intake, ruminant output or environmental benefits cannot be measured directly. Furthermore, some candidate cultivars with proven superior VCU fail DUS even though the non-distinct comparison is with a significantly lower performing registered cultivar. To resolve these problem cases, a "vmDUS" distinctness tool is proposed, which uses molecular markers but conforms to UPOV-declared principles. A short overview of current grassland research shows smart proxy measures of animal value can easily and quickly be adopted into an integrated pan-European (EU-VCU) test network. The proposed EU-VCU scheme will reallocate test resources to conduct these additional tests by placing MS in data sharing collaborations, while retaining their national listing authority. The benefits to all stakeholders from adopting these new testing procedures are considered.

KEYWORDS
cultivar, distinct, uniform and stable, herbage, markers, value for cultivation and use

1 | INTRODUCTION

Before new herbage cultivars can be sold in any EU member state (MS) and gain EU-wide marketing, they must pass official tests to prove they are both novel and improved. Two independent regulatory systems are employed. Plant breeders' rights (PBR) protects existing registered cultivars from plagiaristic exploitation. Value for cultivation and use (VCU) testing requires evidence of a clear value improvement in candidate cultivars compared with existing similar types. Once listed in any MS, a cultivar is automatically included on the EU common catalogue (CC) and has unrestricted marketing rights across the entire EU land mass. The overriding objective of these two schemes for herbage breeding is to stimulate progressive genetic improvement in herbage production that contributes more to ruminant produce while ensuring breeders gain the appropriate market rewards by protecting their improved cultivars when registered.

1.1 | Ruminant production challenges

Grassland farming is currently facing major challenges to its environmental credentials as well as concerns over the financial sustainability of such enterprises and reported negative human health implications of their ruminant products. These issues are global as emphasized by the recent UN, Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES, 2019), with the Intergovernmental Panel on Climate Change (IPCC) placing a high priority on reducing methane and nitrous oxide losses to maintain greenhouse gas (GHG) emissions within planetary boundaries. Livestock are responsible for 14.5% of the global anthropogenic GHG emissions (Gerber et al., 2013), with beef and milk production accounting for 41% and 20% respectively (www.fao.org/3/i3437e). Nearly 40% of EU GHG emissions are of enteric methane, and in 2015, parliament adopted new 2030 target reductions in NH_4 and N_2O emissions from agriculture of 5%–30%, putting ruminant farming in frontline.

There are also considerable financial downward pressures on ruminant-based enterprises selling into price-sensitive markets. This may become more acute as cheaper plant-based analogues for meat and milk are becoming more available (RIIA, 2019) and increasingly being purported as both healthier and less environmentally damaging (Springmann et al., 2018).

In response to these challenges, there is good evidence that ruminant production that is pre-dominantly pasture-fed optimizes the economics of production (Dillon, 2006 and Hanrahan et al., 2018) and provides a product with enhanced flavours that many consumers consider as superior (Yayota, Tsukamoto, Yamada, & Ohtani, 2013). Furthermore, aspects of the environmental loading assumptions have been challenged by more holistic approaches of workers such as Bernués et al. (2017) and estimates of the global carbon sequestration potential of grasslands (Abberton et al., 2010, Ghosh & Mahanta, 2014 and Bouwman, Boumans, & Batjes, 2002). Furthermore, as a major part the fossil energy used in livestock farming is to synthetize and handle mineral nitrogen fertilizers and process concentrate feed, these are all areas where cultivars with enhanced animal value traits could optimize herbage use and contribute significant benefits.

1.2 | Testing system responses

EU herbage cultivar evaluation systems need to actively promote and reward breeding progress that addresses the core challenges. To not respond could marginalize VCU testing schemes and the associated seed production sectors in the priorities of EU policymakers and the wider public opinion. Without prompt action, then in a worst-case scenario, the schemes could become regarded as contributors to a decline in the ruminant sectors they are designed to enhance. Therefore, enhanced VCU systems are now required to identify and promote herbage cultivars that improve nutrient use efficiency through better ruminant utilization, to enhance the environmental positives of grassland farming. Likewise, greater dependence on legume N fixation to reduce fossil fuel inputs now requires VCU testing to examine the dynamics of grass/legume swards to identify legume cultivars with improved competitive ability and nitrogen delivery to sward productivity.

Some examples of such changes in approach already exist. In Ireland, a “Pasture Profit Index” has been developed to reinterpret the standard VCU performance test results and identify those perennial ryegrass (*Lolium perenne* L., PRG) cultivars that best match the seasonal and nutritional demands of spring calving dairy herds (O'Donovan et al., 2016). This promotes better grazing utilization, and a new “grazing efficiency” parameter has been developed for future inclusion in the evaluation system (Tubritt, Gilliland, Delaby, & O'Donovan, 2018). Furthermore, in 2014 France sought to promote the importance of environmental preservation within the evaluation of cultivars, by evolving a VCUE (value for cultivation, use and environment) evaluation concept (Masson & Leclerc, 2014). This gave a higher ranking to cultivars with greater environmental credentials. However, these are individual examples and arguably a more systemic change across the entire EU is required to provide enhanced VCU procedures that better assess each cultivar's efficiency potential when consumed by ruminants. Proposals for what changes are required and how they can be implemented within the EU regulatory systems are expounded in the following sections.

2 | THE vmDUS PROPOSAL

It is vitally important that the PBR system does not pose an impediment to breeding progress when protecting the intellectual property rights (IPR) of registered cultivars.

2.1 | EU-coordinated herbage PBR test procedures

IPR protection for plant cultivars was introduced as a corollary of the post-World War II measures to promote agricultural trade and prioritize food security (Perren, 1995). The international PBR guidelines (established by UPOV in 1961) employ morphophysiological examinations to establish whether candidate cultivars are distinct (uniform and stable—DUS testing) from all existing registered cultivars as a pre-requisite for registering candidate cultivars. Currently, 75 independent countries are signatories to the UPOV convention (www.upov.int), including the EU and its MS. All PBR methods in the EU comply with test procedures set by

TABLE 1 CPVO morphophysiological characteristics used for DUS testing of perennial ryegrass and lucerne cultivars

CPVO Code	Perennial ryegrass Character name (UK)	CPVO Code	Lucerne Character name
2	Plant: vegetative growth habit (without vernalization)	1	Plant: growth habit in autumn of the first year
3	Leaf: intensity of green colour (without vernalization)	2	Plant: natural height 2 weeks after the first autumn equinox following sowing
4	Plant: width (after vernalization)	3	Plant: natural height 6 weeks after the first autumn equinox following sowing
5	Plant: vegetative growth habit (after vernalization)	4	Plant: natural height in spring
6	Plant: height (after vernalization)	5	Time of beginning of flowering
7	Leaf: intensity of green colour (after vernalization)	6	Flower: frequency of plants with very dark blue violet flowers
9	Plant: tendency to form inflorescences (without vernalization)	7	Flower: frequency of plants with variegated flowers
10	Plant: time of inflorescence emergence (after vernalization)	8	Flower:frequency of plants with cream, white or yellow flowers
11	Plant: natural height at inflorescence emergence	9	Stem: length of the longest stem at full flowering
12	Plant: growth habit at inflorescence emergence	10	Plant: natural height 3 weeks after 1st cut
13	Flag leaf: length	11	Plant: natural height 3 weeks after 2nd cut
14	Flag leaf: width	12	Plant: natural height 3 weeks after 3rd cut
15	Flag leaf: length/width ratio	13	Plant: natural height 3 weeks after 4th cut
16	Plant: length of longest stem, inflorescence included (when fully expanded)	14	Plant: natural height 2 weeks after the second autumn equinox following sowing
17	Plant: length of upper Internode	15	Plant: natural height 6 weeks after the second autumn equinox following sowing
18	Inflorescence: length	16	Plant: tendency to grow during winter
19	Inflorescence: number of spikelets	17	Resistance to *Verticillium alboatrum*
20	Inflorescence: density	18	Resistance to *Ditylenchus dipsaci*
21	Inflorescence: length of outer glume on basal spikelet	19	Resistance to *Colletotrichum trifolii*
22	Inflorescence: length of basal spikelet excluding awn	20	Resistance to *Phytophthora medicaginis*
		21	Resistance to *Acyrthosiphon kondoi*
		22	Resistance to *Therioaphis maculata*

the technical committees of the Community Plant Variety Office (CPVO), and harmonized to UPOV guidelines (www.upov.int/resource/en/dus_guidance.html). However, each MS retains its autonomy by making independent listing decisions for their own "National list" (NL). Gaining access to a MS NL affords automatic listing on the EU CC and so EU-wide protection, as part of the EU reference collection used in DUS tests.

UPOV/CPVO guidelines specify which morphophysiological characters are assessed on each species, the assessment methodology, number of test years and the statistical procedures employed. To gain PBR, each new candidate cultivar must be proven distinct (at the $p < .01$ over two testing cycles) in at least one character in every one-to-one comparison with each registered cultivar in the EU CC (the protected reference collection). For example, CPVO-approved examination characters are shown for PRG (CPVO, 2019a) and lucerne (*Medicago sativa*; CPVO, 2019b: UPOV, 2005) in Table 1. Departure from these character sets is strictly curtailed, requiring prior CPVO approval and if particularly novel, also UPOV agreement.

TABLE 2 Distinction occurrences in pair comparisons between 11 lucerne cultivars

		Number of pairwise cultivar comparisons		
Discrimination characteristics	Distinctness analysis (55 pair comparisons)	Not Different	Different in 1 character	Different in > 1 character
Morphophysiological	ANOVA	12	14	29
SSR marker	Allele present/absent	0	8	47
SSR marker	ANOVA on principle components	8	19	28
SNP marker	Frequency difference	26	13	16
SNP marker	ANOVA on principle components	3	8	44
SNP marker	Discriminant analysis	12	-	43

Note: Genotyping performed on 3 independent bulked DNA samples of 100 genotypes per cultivar. Reproduced from Annicchiarico et al., 2016.

UPOV/CPVO-coordinated PBR is regarded as a highly successful IPR scheme, rejecting cultivars that are not adequately distinct from existing protected ones, while also conferring new protection to novel cultivars every year. However, satisfying these distinctness requirements is becoming increasingly challenging in major perennial forages such as lucerne and PRG, owing to the high and ever-increasing number of registered cultivars (currently hundreds per species) and the high within-cultivar morphophysiological variation relative to between-cultivar variation (Annicchiarico, 2006; Julier, Huyghe, & Ecalle, 2000).

For example, the overall rejection rate for the grasses and lucerne in the French NL peaked at 20%–25% after a standard 3-year examination, with around a third of these candidates requiring 1 to 2 extra years of test to overturn the initial refusal (Gensollen, GEVES, pers. comm., 2015). In the UK, candidate rejection rates for *Lolium* spp. and white clover (*Trifolium repens* L.) are around 20%, comprising 12% not distinct and 8% not uniform (Gilliland & Gensollen, 2010; Gilliland, pers. comm., 2019), though Italian ryegrass (*L. multiflorum* Lam.) can be the more problematic species as the gene pool is less diverse.

In general, however, non-distinctions are more frequent between cultivars from the same breeding programme or with contemporary market leaders. This does not indicate any malpractice but rather that breeders can use a leading cultivar (owned by themselves or another breeder) in crosses or for parent plants in crosses with their own breeding pool, to exploit positive genes. However, during the selective crossings or plant evaluations to produce a new synthetic for the same market niche, the new candidate may not sufficiently diverge from that protected leading cultivar, and so correctly fail the DUS test. DUS rejections are a concern, however, when candidate cultivars are excluded from the marketplace by a DUS test that fails to establish a distinction from a registered cultivar that is a significantly poorer performer. UPOV's stated mission is to "provide and promote an effective system of plant cultivar protection, with the aim of encouraging the development of new cultivars of plants, for the benefit of society." In principle, therefore, if a candidate is a statistically significant improvement in VCU to a registered cultivar, and is shown not to be pre-dominately derived from that cultivar, the DUS system should award PBR. To not do so is impeding genetic gain and unjustly penalizing breeders, farmers and agriculture.

2.2 | Potential of molecular markers for cultivar identity in UPOV systems

The emergence of high-throughput genotyping has opened new possibilities for diversity analyses (Byrne et al., 2013) and major gene identification (Liu & Yu, 2017) which offers new strategies for herbage DUS testing (Annicchiarico et al., 2016). Several thousand molecular markers can be obtained in forage species with genotyping by sequencing (GBS) (Elshire et al., 2011). Typically, costs are currently around €35-100/cultivar, depending on methodology and number of reads (Annicchiarico, Nazzicari, Wei, Pecetti, & Brummer, 2017; Byrne et al., 2013).

The ability of such markers to structure a collection of cultivars has been reported in grasses (Pembleton et al., 2016) and legumes (Lucerne, Julier et al., 2018). Furthermore, the successful use of GBS-generated markers from bulked plants to distinguish herbage cultivars has been reported for PRG (Byrne et al., 2013) and lucerne (Annicchiarico et al., 2016; Julier et al., 2018). In Table 2, a published comparison between morphophysiological and molecular markers shows that the molecular-based discrimination of lucerne cultivars was a more stringent tool, particularly when associated with appropriate statistics (Annicchiarico et al., 2016). This study and that of Julier et al. (2018) also revealed a positive but crucially loose relationship between the molecular and morphological diversity. Roldan-Ruiz et al. (2001) also report higher discrimination by genotype examination of PRG cultivars and differing distance estimates, in comparison with standard UPOV DUS testing. So, these general markers are not a proxy for the morphophysiological DUS characters, but provide independent measures of genetic difference unrelated to a cultivar's DUS phenotype.

Molecular markers can, if completely linked to a UPOV-approved morphophysiological character, be used as an alternative descriptor, though none currently exist in herbage species. Presently, UPOV accepts some molecular data, together with morphological traits, to structure registered cultivar collections and, through the “Distinctness Plus” criteria, decide that a candidate is sufficiently dissimilar from a defined part of the reference collection (UPOV, 2013) and de facto distinct from all registered cultivars in that subsection. In some MS, a genotypic difference is used to validate multiple morphophysiological differences that are significant at $p < .05$ (Gensollen, GEVES, pers. comm., 2015). Furthermore, UPOV and the International Seed Federation (ISF) accept a Jaccard coefficient of 0.6 or greater (using SSR markers on bulked plants; Roldan-Ruiz et al., 2000) to implement UPOV’s EDV concept for PRG (ISF, 2009). This use is limited to providing breeders with a metric of relatedness between newly and previously registered cultivars to indicate whether any IPR/royalty sharing agreement is required to permit marketing of the new entity.

Despite evidence of the potential efficacy of marker-based DUS testing, such as that presented to UPOV’s Biochemical and Molecular Techniques working group for lucerne (Julier et al., 2017), UPOV opposes the widespread use of molecular marker-based differences to award PBR for any species. This largely resides in its primary concern to protect the IPR of existing registered cultivars and currently considers that their phenotypic-based identities have primacy. The underlying concern, shared by UPOV and breeders, is the risk that a registered cultivar’s molecular identity could be altered without changing its agronomic value and so be plagiarized by a commercial competitor. This risk is greatly minimized, but not eliminated by using a high number of markers in the discrimination test. The proposal in the current paper is to implement a new “vmDUS” test (as described in 2.3), which totally eradicates the plagiarism risk by requiring the candidate cultivar to have demonstrated a clear improvement in its VCU compared with the registered cultivar.

2.3 | The “vmDUS” concept for avoiding DUS rejection of VCU-improved cultivars

In recognizing the position of principle taken by UPOV, and in line with the other UPOV accepted uses of molecular tests, the vmDUS proposal (value-molecular DUS) seeks to implement a molecular discrimination subject to precisely curtailed VCU (“value”) requirements. This test would only occur when a candidate cultivar has a significantly improved VCU trait compared with a registered cultivar for which the existing DUS procedures cannot provide a $p < .01$ distinction. This specific circumstance would permit a vmDUS examination which, if distinction is achieved, would form part of the description of a new “significantly VCU-improved” cultivar. If the blocking registered cultivar was not in the VCU test with the candidate, a test for significance can be achieved by the long-standing fitted constant statistic defined by Yates (1933) and adapted by Silvey (1978) to provide standard errors for each cultivar pair comparison using linkage controls.

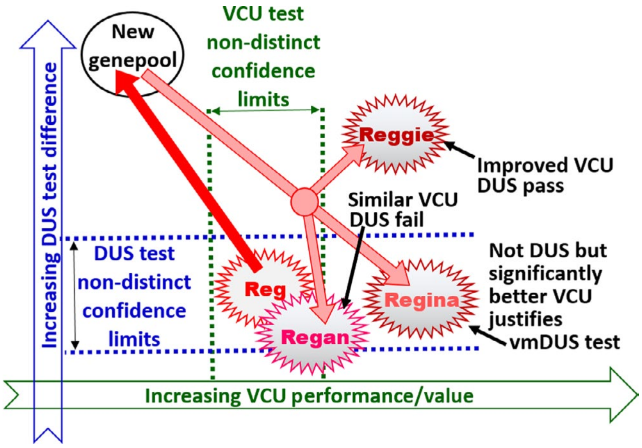

FIGURE 1 Scenario A: divergence—candidate and registered cultivar DUS and VCU relationships. Reg is the registered cultivar that has contributed entirely or largely to the new gene pool to produce candidate cultivars Regan, Reggie and Regina. Broad arrows show germplasm source; dotted lines represent significant difference limits for VCU and DUS [Colour figure can be viewed at wileyonlinelibrary.com]

The concept underpinning the vmDUS proposal is that when a registered and a candidate cultivar are indistinct in UPOV characters, this can have one of two fundamentally different causes, either non-divergence or convergence. These juxtaposing scenarios (A and B below) have differing implications for the protection of existing cultivars:

> Scenario A: Candidate is indistinguishable using current DUS tests as it has not sufficiently diverged in its morphophysiological identity from a shared gene pool of a registered cultivar (the registered cultivar may have provided all or the large majority of the genetic base or the parental material used in the selective crosses, from which the candidate was synthesized).
>
> Scenario B: Candidate is indistinguishable using current DUS tests as it has converged in its DUS morphophysiological identity to a registered cultivar, despite being from a gene pool that is largely independent from that of the registered cultivar.

The main aim of the vmDUS proposal is to register agronomically valuable material bred by valid means, such as the examples in Scenario B, and not block breeding progress which is a core UPOV value. The vmDUS assessment would require the performance of a statistically valid test of cultivar distinctness (based on hundreds or thousands of SNP markers), to verify whether the candidate cultivar was bred from a sufficiently diversified gene pool rather than being essentially derived from a protected cultivar. Hence, the vmDUS test would permit registration of a significantly VCU superior cultivar. Figures 1 and 2 explain pictorially the logic within this decision process:

Figure 1 presents Scenario A in which the candidates (Regan, Reggie and Regina) have all been bred from a gene pool largely provided by the registered cultivar Reg. Therefore, they must sufficiently diverge in DUS characters from the source cultivar Reg to gain PBR. Reggie is both DUS distinct and has an improved VCU compared with Reg and would be expected to automatically pass DUS and VCU tests and get NL listing without any need of a vmDUS test (although unrelated to the vmDUS proposal, but worthy of note, if challenged by the breeder of Reg and Reggie's molecular relatedness to Reg was examined, it might be identified as EDV depend). Regan does not have an improved VCU compared with Reg and is not significantly different in DUS from Reg and therefore would correctly be refused listing, with no justification for a vmDUS test. Regina is not DUS distinct from Reg but has a significantly better VCU performance which would justify a vmDUS examination. As Regina was bred out of a gene pool largely provided by Reg, the molecular markers would reveal the degree of relatedness to Reg and would determine whether Regina passed the vmDUS test or failed for being too closely derived from Reg. As described earlier, this would be a UPOV and ISF-approved pass/fail vmDUS threshold derived in the same manner as for the PRG EDV threshold (Roldan-Ruiz et al., 2000 and ISF, 2009).

Figure 2 presents the alternative Scenario B, where all candidates (starter, fresh and initial) are new synthetics from an independent gene pool to that of Reg. In this example, fresh is both DUS distinct and higher performing than Reg and so passes both the DUS and VCU tests to automatically get NL listed. Starter has converged with Reg as it is not DUS distinct from it and has a similar VCU performance. So, it correctly fails DUS with no justification for a vmDUS test (even if its VCU performance was sufficient to pass the minimum NL entry level). Initial is not DUS distinct from Reg but is significantly higher VCU performing. Similar to Regina in Scenario A, evidence of VCU superiority over the blocking cultivar would justify a vmDUS test. In this instance, however, the molecular markers would be expected to reveal a large genetic distance between initial and Reg and thus evidence of phenotypic convergence from a distinct gene pool. Therefore, initial would pass the vmDUS test and get NL listed. This would correctly reward the breeder for achieving a significant genetic improvement by a valid breeding activity and insure it could be marketed to benefit farmers.

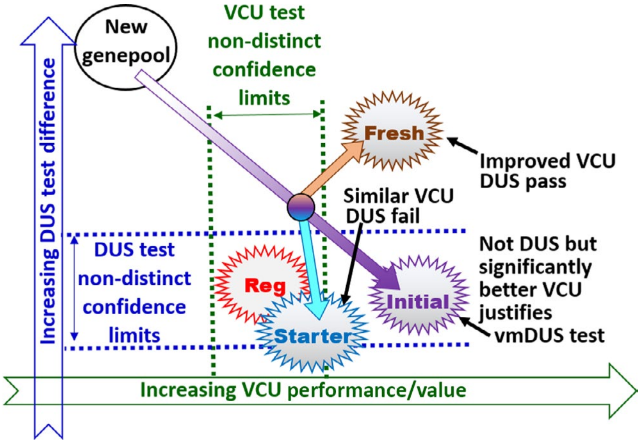

FIGURE 2 Scenario B: convergence—candidate and registered cultivar DUS and VCU relationships. Reg is the registered cultivar; fresh, starter and initial are candidates bred from a largely independent gene pool. Broad arrows show candidate germplasm source; dotted lines represent significant difference limits for VCU and DUS [Colour figure can be viewed at wileyonlinelibrary.com]

2.4 | Implementation of the vmDUS proposal

By a process that matches that of the morphophysiological trait-based distinctness, vmDUS-based distinctness would assess the whole set of available markers and using a statistical test to express a type 1 error probability, apply a $p < .01$ pass threshold. As achieved by Annicchiarico et al. (2016), the proposed test could be based on 3–4 independent bulked DNA samples each from a separate set of 150–200 plants, applying ANOVA of cultivar scores on principal component axes or discriminant analysis. As an alternative, distinctness could be granted on the grounds of a minimal threshold of a genetic distance measure (UPOV, 2013), without statistical tests. Indeed, for species where there is an agreed molecular threshold for EDV, a UPOV/ISF-approved pass standard for the vmDUS test could quickly be set beyond the EDV threshold, by using the same research evidence.

To implement vmDUS within the EU for any herbage species will require the prior genotyping of the entire reference collection and the provision of an easy-to-use marker tool to registration offices, comprised of some hundred highly discriminating SNP markers (such as a small array or a RAD capture tool). Currently, two H2020 projects, INVITE and InnoVar, are scoping the definition of such a tool for lucerne and PRG.

Although vmDUS would be the first time that VCU data were considered in awarding PBR, when the current DUS system blocks the registration of significantly higher performing candidates, it infringes the core UPOV objective of promoting genetic improvement. As morphophysiological DUS character testing cannot differentiate divergence from convergence, molecular markers are the only option to resolve this unacceptable anomaly.

3 | THE EU-VCU PROPOSAL

This proposal aims to augment the current VCU testing of herbage cultivars across EU MS, in order to reward breeders for addressing the wider challenges facing the ruminant production sectors. This means evaluating herbage traits that can promote higher livestock production efficiency, higher positive environmental impact and greater consumer acceptability of ruminant produce. There are two key hurdles to be overcome.

- Identify additional traits that can be measured on large candidate numbers in a cost- and labour-efficient way.
- Devise a means of reassigning existing resources to conduct these additional evaluations from within the existing independent MS VCU schemes.

This EU-VCU proposal aims at resolving both these issues through an EU MS data sharing scheme that implements a division of labour and adoption of new test parameters. In practice, this would involve pooling test resources between MS sharing an agrizone (as described in section 3.4.1). All the current VCU parameters could still be recorded plus newer animal performance/environment-focused characters (as identified in sections 3.31 and 3.32) could be added by redistributing tasks across a larger number of test sites.

3.1 | EU MS VCU test procedures

There is no single EU-coordinated VCU testing system. Independent grass and legume VCU test procedures exist in each MS and are designed to reflect their farming practices, growing conditions and the importance of each species in that MS. The main parameters measured are nonetheless largely similar, though their relative importance can differ greatly. Likewise, the baseline elements involve multisite, multiple year testing to obtain an accurate mean comparison between each candidate and pass/fail standards set using exemplar national control cultivars.

The performance characters typically include annual and seasonal DM production (by plot harvester), herbage quality (in vitro) and persistence. MS protocols differ mainly in levels of applied nitrogen, sward use (grazing—Gz, or cutting—Cn), cutting height/frequency, timing of quality sampling and disease exposure procedure. These tests are intended as indicators of animal performance potential as herbage yield drives intake levels (Dillon, 2006) and chemical analysis provides indirect estimates of "appetibility" and "ingestibility" (e.g. Gillet & Jadas-Hécart, 1965) and milk or meat production (Alothman et al., 2019). Likewise, disease resistance can be regarded as an "animal" attribute, as high tolerance protects herbage yield and green leaf-driven intakes (Woodfield & Easton, 2004).

Further differences between MS exist in the diseases and degree of pressure that exists. Therefore, the required cultivar resistance differs between MS, resulting in a different stringency of testing being imposed. The type and amount of VCU performance data collected is also reduced if a species is designated as minor in a MS. For example, PRG, cocksfoot (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.) and lucerne are major species in France and tested on at least six sites. For minor species, there are only three test sites, including the breeder's location. Similarly, the UK tests PRG at six sites and minor species such as Timothy (*Phleum pratense*, L.) and Italian ryegrasses (*L. multiflorum*, Lam.) at three sites, with no grass quality measured (Anon, 2019). The number of annual sowings and test years also ranges from single to multiples depending on the importance of a species to a MS's ruminant milk and meat production sectors.

3.2 | Limitations of "Detached" VCU testing within MS

The most obvious anomaly in VCU testing is that MS often report substantially different disease and climatic stress tolerances for the same cultivar. This cultivar GxE interaction is widely recognized. For example, Annicchiarico, Barrett, Brummer, Julier, and Marshall (2015) reported low and occasionally negative genetic correlations (r_g) for lucerne or red clover across drought-prone and moisture-favourable conditions (r_g ranging from −0.34 to 0.66; average r_g = 0.10). So, a cultivar could enter the NL in one MS with good disease or climatic tolerance scores but fail to pass the minimum standards under more challenging conditions in another MS. Despite this, that cultivar would enter the EU CC and gain the right of sale in the second MS. Likewise, a successful cultivar VCU test in one MS, where it is a minor species, can open access to other MS markets where it is a major species without having been tested to the same level of accuracy as cultivars submitted to that territory. This, therefore, avoids the higher pass threshold and is an acute shortcoming of the detached NL systems of MS in the EU. An implication of this is that extension services often limit their recommendations to cultivars that have been listed as major species on their own MS NL. This is because they are assured that the VCU performances have been achieved under the stress conditions within their region. However, this somewhat undermines to CC concept on a uniform EU marketplace. These inequalities can be successfully redressed by the new EU-VCU proposal.

3.3 | Cultivar attributes for an enhanced EU-VCU

The key objective in herbage VCU testing is to predict the animal performance each cultivar can potentially support, in order to inform herd feed management plans. As herbage allowance drives animal intake (Dillon, 2006), biomass yield over the season will remain a primary VCU trait, as will herbage quality, sward longevity and tolerance to major climatic and biotic stresses. However, as evidenced earlier, current ruminant production challenges also require assessment for additional parameters that reduce N excretion and GHG emissions and also impact on the human health value of meat and milk, by identifying cultivars that achieve a more efficient animal utilization and resource input use. Examples of currently reported characteristics that can assess these required attributes are as follows:

3.3.1 | Animal performance promoters

Simulating grazing at low and frequent cutting does not fully represent how grazing animals perform on cultivars (Byrne et al., 2017), as differences in factors driving voluntary intake and grazability have a significant modifying effect. As directly measuring animal performance is too costly and labour-intensive, it has been proposed that the only option is to measure "indirect grazing predictors" and "predictors taken under mob grazing" (Gilliland, Hennessy, & Ball, 2018).

Available indirect PRG grazing predictors include "free leaf lamina" (FLL; Cashman, 2014 and Wims, McEvoy, Delaby, Boland, & O'Donovan, 2013), or "sward leaf content" (Beecher et al., 2015 and Flores-Lesama, Hazard, Betin, & Emile, 2006), longer growth from

TABLE 3 Improved silage digestibility reducing concentrate use and nutrient and GHG losses in Northern Ireland (NI)

	Dairy production (67% D silage 140 days)		Beef production (62% D silage 150 days)	
	+5 Units D	+10 Units D	+5 Units D	+10 Units D
Silage feeding in winter period (days/yr)	140	140	150	150
Less P input: reduced conc./head (kg/winter)	1.10	2.20	1.29	2.58
Less P excreted: saved conc. in NI (t/winter)	103	205	117	233
Less N excreted: lower total diet intake/head (kg/winter)	1.92	3.84	–	–
Less enteric CH_4 emission: lower total diet intake/head (kg/winter)	1.4	2.7	–	–

Note: Values compiled from report by T. Yan (personal communication) using data from multiple studies and interpolated from silage quality analyses. Values based on an 80% silage utilization and a crude protein concentration in the total diet of 170 g/kg DM.

spring to heading ("Flexibility for management," Tabel & Allerit, 2005) and differences in DM content (Meehan & Gilliland, 2019). Predictors taken under mob grazing include post-grazing sward height (Tubritt et al., 2018) and reduced secondary heading (O'Donovan & Delaby, 2005). Importantly, PRG flag–leaf length in DUS spaced plants is a strong predictor of FLL through the growing season and DM content is routinely measured in VCU tests but currently not reported. So, all these example parameters represent some of the readily available indicators of animal intake potential. Incorporating some or all of these into an enhanced VCU scheme can be expected to drive higher animal productivity by, for example, 1.6 kg milk/cow/day from grazed grass across existing FLL differences (Cashman, 2014; Wims et al., 2013) or enhance intake by 1 kg DM per 40 g/kg for existing DM content differences (Vérité et al., 1970). Furthermore, near-infrared spectrometry (NIRS) now offers rapid and low-cost chemical analyses on large sample numbers that can be used to predict livestock responses and rank cultivars. Calibrations based on in vivo digestibility studies in sheep (Zhao, Annett, & Yan, 2017), growing cattle (Hynes, Stergidias, Gordon, & Yan, 2016) and dairy cows (INRA, 2018; Agnew et al., 2004) can be translated into meat and milk productivity. Animal performance can also be estimated by proxy for other nutritional factors. For example, a fall in grass protein content below 12% aligns with a 2 kg DM reduction in daily herbage intake and a 2.5 kg reduction in milk yield (Peyraud & Astigarraga, 1998). Similarly, a 4% increase in lucerne digestibility equates to an extra 1.4 kg of milk/day (Emile, Génier, & Guy, 1993) and cultivar differences in quality have been confirmed (Julier et al., 2001). Likewise, differences in grass WSC correlate strongly with changes in net energy (Lila, 1977) and rapid silage fermentation with improved rumen efficiency when grazed (Miller et al., 2001) or fed ensiled (Merry et al., 2006). Current VCU testing for nutritive value has raised digestibility annually by 0.5–1.0 g/kg DM (McDonagh, O'Donovan, McEvoy, & Gilliland, 2016; Wilkins & Humphreys, 2003) and indicates what could be achieved if MS test centres had access to the full suite of quality parameters for every herbage species. Similarly, IVDMD in perennial ryegrass was found to have annually increased by 0.39 g/kg DM among the varieties registered on the French NL from 1971 to 2003 (Sampoux et al., 2011). As many of the aforementioned studies involved farm-scale or "farmlet"-level experiments, the potential practical benefits that commercial enterprises could gain by VCU testers adopting these proxy animal performance predictors are clearly demonstrated and quantified.

3.3.2 | Environmental footprint protectors

Cultivar parameters that improve herbage production and ruminant ingestion also lower the emission intensity (cost per unit of product). Although not a simple relationship (Negussie et al., 2017), a 10% lower GHG emission from dairy cows and 17% less per beef carcass, due to improved grass quality for silage, has been reported by Bente, Randby, Bonesmo, and Aass (2019). This was largely by replacing concentrates that incur a much higher GHG cost during manufacturing and transport. Furthermore, Table 3 summarizes multiple studies using indirect open-circuit respiration calorimeters, and shows that improved silage quality fed to dairy and beef animals reduced concentrate use, and so phosphate and nitrate excretion and lowered methane emissions. Other options for cultivar testing include measuring condensed tannin levels in sainfoin (*Onobrychis viciifolia*) and bird's-foot trefoil (*Lotus corniculatus*) to reduce rumen protein degradation and urine N excretion (Theodoridou et al., 2010), or saponins in many legumes including lucerne, to reduce ruminal degradation by protozoa and lower methane emissions (Niderkorn & Baumont, 2009). Likewise, testing grass cultivars for differences in nitrogen absorption and legumes for N fixation rates could contribute to reducing losses into waterways from pasture land.

As cultivar ranking in pure stands does not always translate to the same ranking in mixed stands (Annicchiarico, 2003; Maamouri, Louarn, Béguier, & Julier, 2017), herbage species that are frequently grown in mixtures would be better tested also (or only) in mixed

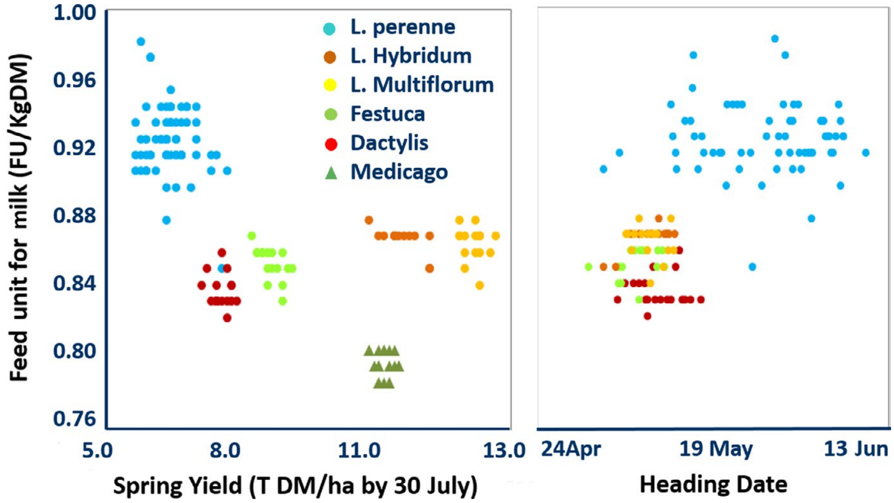

FIGURE 3 Feeding value of grass and legume species/cultivars from the French NL since 2010 (www.herbe-book.org). Spring yield is all cuts before July 10 in years 2 and 3 from 5 to 8 locations. Feed unit for milk is calculated from the weighed ADF content in the first three cuts year 1. One feed unit refers to 1 kg of barley as 1,700 kJ/kg DM of net energy [Colour figure can be viewed at wileyonlinelibrary.com]

stands, as done, for example, for white clover (*Trifolium repens*, L.) cultivars in the UK (APHA, 2019). A number of studies reviewed in Annicchiarico et al. (2019) indicate that the inconsistency for cultivar yield response across pure stand and mixed stand is larger (thereby justifying greater attention) for species with poorer competitive ability. To maintain a reasonable evaluation cost, a control mixture of species/cultivars, appropriate to the candidates' common use on farm, could be used to test for general compatibility, which is justifiable as specific-compatibility effects are reportedly smaller than general compatibility ones (Annicchiarico et al., 2019). On the breeding side, recent theoretical expectations suggest feasibility and effectiveness of a selection for mixture usage by controlling the direct and associate effect among progenies within each partner or species (Sampoux, Giraud, & Litrico, 2020). Cultivars that stabilize their proportions in mixed stands would help reduce herbicide use, improve consistency of herbage production, reduce inorganic nitrogen use and/or provide a better protein to energy balance for greater dietary N absorption (Niderkorn & Baumont, 2009).

Organic farming is extending across Europe and thus must be taken into account within the cultivar VCU assessments. Forage production is not far from organic farming except that chemical nitrogen is used to fertilize grasses, and herbicide is used on lucerne fields at establishment. Given the evidence of Wilkins, Allen, and Mytton (2001) and AHDB (2016) that cultivar ranking was conserved in PRG and Timothy across an N fertilizer range of 100–500 kgN/ha, they conclude that recommendations from high N VCU trials can be applied with some confidence to lower N practices. For pure legumes, additional assessment without herbicide does not seem justified on the basis of the consistent yield response of lucerne cultivars across conventional management (subjected to chemical weeding) and organic management mimicked by severe weed invasion (Annicchiarico & Pecetti, 2010), which arose from the fact that higher yield in the absence of weeds and higher competitive ability against weeds depend both on higher relative growth rate of the cultivars. However, both aspects can also be resolved by converging the growing of mixed stands to include the assess of cultivar suitability for organic conditions, as described above.

3.4 | The EU-VCU concept of agrizones and resource sharing

A key role for new cultivars is to support farmers in achieving a profitable and sustainable business. Typical estimates of the gross potential value that cultivars with higher animal utilization potential range around €230-380/ha/year (dairy) or €180-250/ha (beef) per additional tonne ingested (DAERA, 2016) or an additional €181 net profit/ha (Hanrahan et al., 2018). Similarly, the estimated range in animal performance among all grass and lucerne cultivars presently registered in the French list is shown in Figure 3 as "feed unit for milk." The highest range of feeding value, 0.12 feed units, was found in PRG with no correlation with yield in spring or earliness. These financials highlight the need for additional VCU tests.

Although a large number of suitable proxy tests have been identified to promote cultivars that support better animal performance, environmental impact and human health benefits, none of these can be adopted without extra resources. As applicant breeders usually pay all or part of the VCU costs and strongly oppose fee rises, national testing authorities work under strict financial limits. This currently curtails what tests are conducted and so obtaining additional funding to substantially enhance VCU testing is highly unlikely. Therefore, core to the EU-VCU proposal is data sharing and division of tasks across MS to reallocate resources.

This EU-VCU concept proposes to establish "agrizones," each defined as a specific edaphic, environmental and agronomic region. These comprise of land pockets of a common type and extend across MS borders. MS will collaborate within these agrizones by pooling their combined test sites to redistribute the workload of conducting both the standard and additional "proxy" VCU tests. This collaborative approach provides MS with access to additional testing resources, but retains each MS's own national listing authority. There are clearly two practical challenges to conducting such collaborations, namely the "establishment of the data sharing agrizones" and "procedures for integrating data" from different MS testing schemes, without imposing a strict uniformity of testing method.

3.4.1 | Establishing agrizones

Identifying agrizones is readily achievable as in the agro-climatic zone approach by van Wart et al. (2013) used to predict crop yield potential. As reported by Jongman et al. (2006), it is now possible to compile continent-wide statistically based environmental stratifications that can be applied consistently throughout Europe, as evident in Figure 4. The agrizone demarcation would be species by species, although much commonality is expected among the herbage species. Crucially, it would depend on location ordination and classification according to GxE interaction effects for yield using multisite data from a relevant and broadly based set of reference cultivars overlaid with disease and climatic stress patterns (Annicchiarico, 2002). Due to its withdrawal from the EU, the UK will likely not partake in any EU agrizone scheme and so the ATN and ATC climatic regions (Figure 4) would be reduced in scope. Powerful statistical procedures such as AMMI or factorial regression analyses (Gauch, 1992) can be used to model cultivar responses to key environmental and edaphic factors. As climatology has made huge progress in prediction modelling for climate change monitoring, this will make agrizone classification more precise. Likewise, where a MS employs a managed testing environment for climatic or disease stress testing (as for lucerne, Annicchiarico & Piano, 2005), this could be shared with another MS in an agrizone or be rendered unnecessary if these stresses frequently occur naturally in that other MS. As each agrizone will be a data capture region with listing decision remaining within each MS, authorities can independently apply appropriate weightings and interpretations to rank varieties for adaptation to their own regional farming systems. This would be particularly expected in agrizones that span widely dispersed land masses, such as ATC and MDM (Figure 4).

3.4.2 | Integrating data

When examined in detail, differences between national VCU schemes are apparent, but all follow similar processes and assess largely the same characters in a given species. These differences reflect regional best practices and need not preclude data sharing for a common goal of ranking cultivars for each VCU character, under incident conditions. So, although greater harmonization will likely evolve over time as best practices become apparent, EU-VCU does not need to impose a prescribed VCU procedure within an agrizone, but integrates data from different autonomous MS testing schemes. By sharing common control cultivars, it is possible to transform these data and incorporate the results from each MS site into a shared agrizone database. As already states, MS would apply their own pass/fail standards and policy priorities for their NL. So, where an agrizone was a minor region in a MS, EU-VCU could provide the data needed for major species level decisions, so standardizing NL pass/fail stringency across the EU.

Retaining MS automony is vital as the decision makers in some MS are government officials (e.g. Ireland, Germany—Federal and

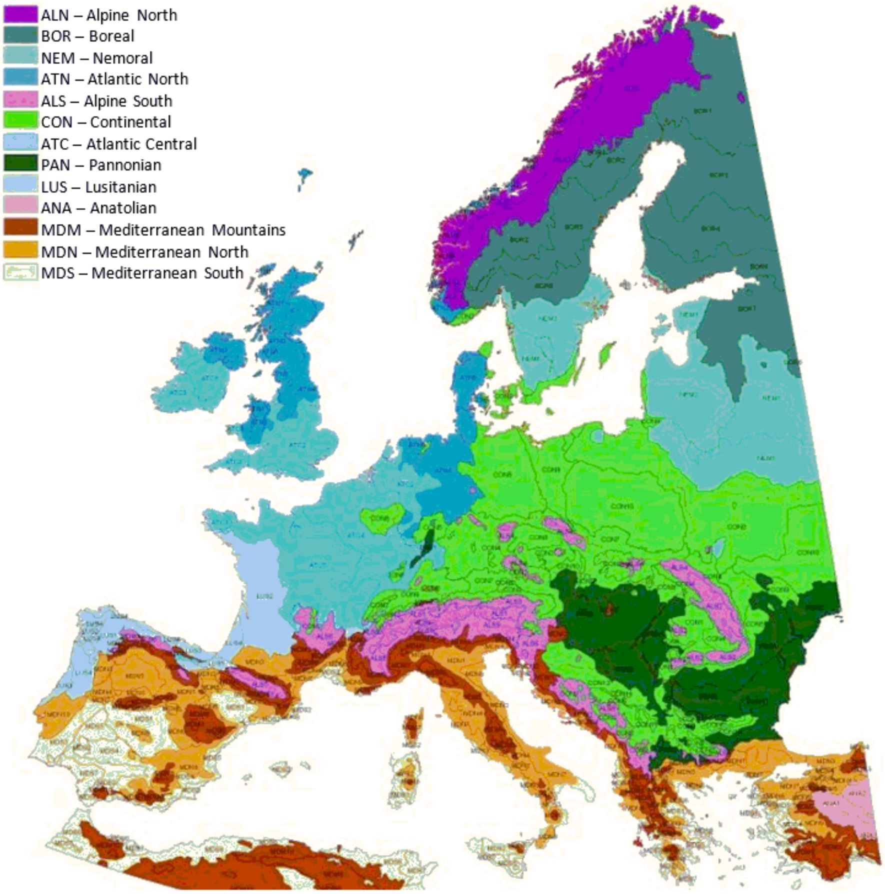

FIGURE 4 The environmental stratification of Europe, after Metzger, Bunce, Jongman, Mücher, and Watkins (2005), modified copy retrieved from https://www.researchgate.net/publication/235437977 [Colour figure can be viewed at wileyonlinelibrary.com]

Bundesländer), but in the Netherlands, Wageningen University oversees a consultancy group of industry, breeders, end users and testers. In France, the Ministry of Agriculture takes account of a consultative committee (Permanent Technical Committee of Selection) comprising breeders, public representatives and end users. This overlays a diversity of funding streams (official, levy body, fees and industry support) that are often specifically justified to support only the markets within that territory. Hence, retaining these existing structures will make implementing an EU-VCU administratively simple and more achievable. This proposal could also make the EU CC more creditable if cultivars were listed with the defined agrizone that the MS used to enter it on its NL. This would recognize regional growing differences within the EU, without compromising the single market concept.

3.5 | EU-VCU stakeholder commitment

A successful implementation of an EU-VCU scheme will require all stakeholders to be convinced of the benefits. The process will initially require coordination, probably at the EU level, to implement the key steps which are as follows:

- MS agree the cross-border agrizones for a species and the division of tasks for assessing "standard" and "additional proxy" characters, ideally in a workload neutral arrangement.
- MS retain their current protocol for existing "standard" characters within an agrizone.
- Test centres comply with agreed quality assurance standards and best practices, including management of a shared database with each MS having "real-time" full access.
- MS accept to test each other's candidates in their agrizone sites on a full cost recovery basis.
- Some common control cultivars with putative contrasting adaptation patterns are sown at all test sites in an agrizone to integrate results from different MS test systems into the agrizone database.
- MS retain own listing authority, applying their own decision standards and indices.
- EU adopt CC listings that denote the EU agrizone(s) for which each cultivar is adapted.

If two MS protocols differ so widely for a species that using linkage controls alone to combine data would not be effective, then some harmonization of methodology might be required of the test sites sharing data within an agrizone. However, a single unified protocol is still not an essential requirement and variations in MS protocols can be accommodated.

Among the various stakeholders, EU policymakers should readily favour an EU-VCU, as its more pan-EU functionality is a better fit with the single market policy. It also makes the EU CC a more creditable document as it would become not just a conglomerate of MS listings but reflect diversity of conditions and practices within the single market. MS are also beneficiaries, as the data sharing element means they can access resources to test newer characters and demonstrate a proactive response to the rural sustainability, environmental and human health issues that are core policy drivers in all EU MS. Given the quality assurance aspect of the EU-VCU, there should not be any insurmountable legalities or liabilities for MS that accept candidates that are partly tested outside of their national jurisdiction. Likewise, funding (especially levies) can be managed to ensure they benefit the local jurisdiction they are intended to support.

Currently, breeders decide which cultivars to submit to each MS, but with an EU-VCU they can more accurately choose one or more agrizones that align with the breeding criteria of each candidate cultivar. Whether breeding for broad adaptation or to optimize under specific conditions and cultural practices, this would be attractive to both large corporate and small regional breeders. This system would enable a cultivar to be considered by several MS NL when an agrizone spans across their borders. An EU-VCU would support and reward breeders that are seeking to improve the animal value potential of new synthetics and niche cultivars with specialist attributes are more likely to achieve a VCU pass. Furthermore, some authorities now invite breeders to submit data for some DUS characters (e.g. flower colour, autumn dormancy, disease tolerance), to place the candidate beside similar types and improve the proportion of positive tests, e.g. GEVES for lucerne. Although this practice is confined to only some authorities and only some species, it diverts breeding effort away from selecting for improvements. Although not a UPOV recommended approach, it might become more prevalent and is an impediment to genetic progress. The vmDUS safety net would make this additional burden unnecessary and so further benefit breeders. Breeders will, however, require reassurances that an EU-VCU scheme does not incur either higher test fees or more administrative complexity compared with the status quo.

Seed merchants will appreciate the additional animal performance potential information. This will help them demonstrate the benefits of using these new cultivars and support a quality seed market and pricing structure. The only possible negative impact might be that agrizone-based listings could subdivide some existing seed markets and require additional cultivars to be maintained. However, the benefits should outweigh this more minor consequence.

Farmers are likely to be supportive as they are concerned with the end point recommendation, and if these are more specific to their farming region and more informative about the cultivar's animal performance supporting potential and their contribution to agriculture sustainability, this will be a significant benefit to their farm business.

4 | CONCLUSIONS

While the environmental issues surrounding food production support the need for less impacting, more extensive systems, the pressure of meeting the nutritional needs of the increasing human population is of equal priority. Likewise, the health risks of over consumption of ruminant products must be set in the wider context. Willett et al. (2019) report that over 280 m people are regularly short of adequate food (some estimate this at 800 m) and many more have low-quality micronutrient-deficient diets that cause nutrition-related non-communicable diseases. These pose greater risks of morbidity and death than the combined impact of alcohol, drug and tobacco use and unsafe sex. As the EU-28 (27 when minus UK) produce around 14% of the meat and 22% of the milk production globally, have 85 million sheep on 830,000 farms and were among the largest producers of milk in absolute terms and relative to population size in the G20 in 2016, this nutritional supply must be retained and optimized (Eurostat 2018).

As the PBR and VCU tests are the gateway to the EU marketplace, they must be proactively contributing solutions for the headline issues of ruminant production. First and foremost, PBR must protect the commercial investment imbedded in existing registered cultivars and so candidates that are not "unique" and "improved" are correctly barred from the market. These schemes must also stimulate and promote the release of "unique," "improved" candidates by removing any unjustifiable impediments. This is what the proposed vmDUS system is designed to do, by differentiating between true breeding and plagiaristic exploitation of an existing cultivar. This will ensure the registration of cultivars with superior VCU that is the product of valid breeding activity. The vmDUS test does this without setting a prescient that automatically leads to a wider use of molecular markers. However, in establishing that a significant VCU improvement can justify a vmDUS measure of relatedness in herbage species, the same principle could be sought for other crop species.

Where the terrain of several MS overlaps into similar agrizones, it is reasonable for MS to share data but so far, they do not. Moreover, if a MS has a specific growing area that is too small a region to justify a dedicated national VCU test site, data importing from that agrizone in another MS would be helpful, but do not occur. Paradoxically, once one MS lists a cultivar it can be sold in every EU territory, despite the differing importance of that species and stringency of testing between MS. The EU-VCU proposal seeks to resolve these issues and to also redistribute test resources so that new proxy animal value characters and traits that contribute to sustainability and human health can be examined. These extra resources will not be limitless, and so testers will need to prioritized which traits to fund in each species. Although these new traits do not directly measure the magnitude of animal benefit, they can be applied to large candidate numbers to drive a progressive improvement in this key factor over time.

There is enormous responsibility on evaluators to protect breeders with existing profitable businesses while also promoting breeding progress to enhance growers' enterprises, support rural communities, lower environmental footprints and promote safe and abundant food. Continuing with the current systems unchanged is falling short of what could and should be achieved, but requires political will and intervention at the senior EU-28/27 level, to support the testing authorities in implementing these pan-EU changes.

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REFERENCES

UPOV. (2005). *Lucerne: Guidelines for The Conduct of Tests for Distinctness, Uniformity and Stability*. Document TG/6/5; Geneva, pp 34. Retrieved from https://www.upov.int/edocs/tgdocs/en/tg006.pdf

UPOV. (2013). *Guidance of the use of biochemical and molecular markers in the examination of distinctness, uniformity and stability (DUS)*. Document TGP/15, Geneva, pp 1–10. Retrieved from https://www.upov.int/meetings/en/doc_details.jsp?meeting_id=47214&doc_id=415191

AHDB. (2016). *Varietal testing under reduced nitrogen conditions*. Report prepared for AHDB Dairy (Agriculture and Horticulture Development Board), January 2016, pp 5. Retrieved from https://dairy.ahdb.org.uk/media/1361947/Executive%20Summary%20-%20Varietal%20testing%20under%20reduce%20N%20conditions.pdf

DAERA. (2016). *Delivering our future, valuing our soils: a sustainable agricultural land management strategy for Northern Ireland*. DAERA Occasional Publication. ISBN 978-1-84807-708-9. Retrieved from https://www.daera-ni.gov.uk/publications/sustainable-agricultural-land-management-strategy-report-and-executive-summary

IPBES. (2019). *UN, Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, The global assessment report on biodiversity and ecosystem services*. p. 45. May 2019. Retrieved from https://ipbes.net/global-assessment

APHA. (2019). Animal and Plant Health Agency. *United Kingdom National List Trials: Trial Procedures for Official Examination of Value for Cultivation and Use (VCU) Harvest 2019: White Clover*. pp 27. Retrieved from https://www.gov.uk/guidance/vcu-protocols-and-procedures-for-testing-agricultural-crops

CPVO. (2019a). *Community Plant Variety Office, Protocol for tests on distinctness, uniformity and stability – Lolium*, CPVO-TP/004/2, 19/03/2019. Retrieved from https://cpvo.europa.eu›technical-examinations›technical-protocols

CPVO. (2019b). *Community Plant Variety Office, Protocol for tests on distinctness, uniformity and stability – Medicago sativa*, CPVO-TP/004/2, 19/03/2019. Retrieved from https://cpvo.europa.eu›technical-examinations›technical-protocols

Abberton, M., Conant, R. T., & Batello, C. (Eds). (2010). *Grassland carbon sequestration: management, policy and economics*. In: Proceedings of the workshop on the role of grassland carbon sequestration in the mitigation of climate change, Plant Production and Protection Division, Food and Agriculture Organization of the United Nations FAO, Rome, pp 338, ISBN 978-92-5-106695-9. Retrieved from http://www.fao.org/3/a-i1880e.pdfhttp://www.fao.org/3/a-i1880e.pdf

Agnew, R. E., Yan, T., France, J., Kebreab, E., & Thomas, C. (2004). Energy requirement and supply. In: C. Thomas (Ed.), *Feed into milk: A new applied feeding system for dairy cows* (pp. 11–20). Nottingham, UK: Nottingham University Press.

Alothman, M., Hogan, S. A., Hennessy, D., Dillon, P., Kilcawley, K., O'Donovan, M., ... O'Callaghan, T. F. (2019). The "Grass-Fed" Milk Story: Understanding the Impact of Pasture Feeding on the

Composition and Quality of Bovine Milk. *Foods (Basel, Switzerland)*, *8*(8), 350. https://doi.org/10.3390/foods8080350

Annicchiarico, P. (2002). *Genotype × environment interactions: Challenges and opportunities for plant breeding and cultivar recommendations*. FAO Plant Production and Protection Paper No. 174. Food and Agricultural Organization, Rome, pp 115. Retrieved from http://www.fao.org/3/y4391e/y4391e00.htm

Annicchiarico, P. (2003). Breeding white clover for increased ability to compete with associated grasses. *Journal of Agricultural Science*, *140*, 255–266. https://doi.org/10.1017/S0021859603003198

Annicchiarico, P. (2006). Diversity, genetic structure, distinctness and agronomic value of Italian lucerne (*Medicago sativa* L.) landraces. *Euphytica*, *148*, 269–282. https://doi.org/10.1007/s10681-005-9024-0

Annicchiarico, P., Barrett, B., Brummer, E. C., Julier, B., & Marshall, A. H. (2015). Achievements and challenges in improving temperate perennial forage legumes. *Critical Reviews of Plant Science*, *34*, 327–380. https://doi.org/10.1080/07352689.2014.898462

Annicchiarico, P., Collins, R. P., De Ron, A. M., Firmat, C., Litrico, I., & Hauggaard-Nielsen, H. (2019). Do we need specific breeding for legume-based mixtures? *Advances in Agronomy*, *157*, 141–215. https://doi.org/10.1016/bs.agron.2019.04.001

Annicchiarico, P., Nazzicari, N., Ananta, A., Carelli, M., Wei, Y., & Brummer, E. C. (2016). Assessment of cultivar distinctness in alfalfa: A comparison of genotyping-by-sequencing, SSR marker and morphophysiological observations. *The Plant Genome*, *9*. https://doi.org/10.3835/plantgenome2015.10.0105

Annicchiarico, P., Nazzicari, N., Wei, Y., Pecetti, L., & Brummer, E. C. (2017). Genotyping-by-sequencing and its exploitation for forage and cool-season grain legume breeding. *Frontiers in Plant Science*, *8*, 679. https://doi.org/10.3389/fpls.2017.00679

Annicchiarico, P., & Pecetti, L. (2010). Forage and seed yield response of lucerne cultivars to chemically weeded and non-weeded managements and implications for germplasm choice in organic farming. *European Journal of Agronomy*, *33*, 74–80. https://doi.org/10.1016/j.eja.2010.02.006

Annicchiarico, P., & Piano, E. (2005). Use of artificial environments to reproduce and exploit genotype × location interaction for lucerne in northern Italy. *Theoretical and Applied Genetics*, *110*, 219–227. https://doi.org/10.1007/s00122-004-1811-9

Anon. (2019). *Protocols and procedures for testing the value for cultivation or use (VCU) of agricultural crops*. UK Government. Retrieved from https://www.gov.uk/guidance/vcu-protocols-and-procedures-for-testing-agricultural-crops#procedures--for-2020-harvest

Beecher, M., Hennessy, D., Boland, T. M., McEvoy, M., O'Donovan, M., & Lewis, E. (2015). The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility. *Grass and Forage Science*, *70*, 19–29. https://doi.org/10.1111/gfs.12081

Bente, A. A., Randby, Å. T., Bonesmo, H., & Aass, L. (2019). Impact of grass silage quality on greenhouse gas emissions from dairy and beef production. *Grass and Forage Science*, *74*(3), 525–534. https://doi.org/10.1111/gfs.12433

Bernués, A., Rodriguez-Ortega, T., Olaizola, A., & Bosch, R. (2017). Evaluating ecosystem services and disservices of livestock agroecosystems for targeted policy design and management. *Grassland Science in Europe*, *22*, 259–267.

Bouwman, A. F., Boumans, L. J. M., & Batjes, N. H. (2002). Emissions of N2O and NO from fertilized fields: Summary of available measurement data. *Global Biogeochemical Cycles*, *16*(4), 6–1–6–13. https://doi.org/10.1029/2001GB001811

Byrne, N., Gilliland, T. J., McHugh, N., Delaby, L., Geoghegan, A., & O'Donovan, M. (2017). Establishing phenotypic performance of grass varieties on Irish grassland farms. *Journal of Agricultural Science*, *155*, 1633–1645. https://doi.org/10.1017/S0021859617000740

Byrne, S., Czaban, A., Studer, B., Panitz, F., Bendixen, C., & Asp, T. (2013). Genome Wide Allele Frequency Fingerprints (GWAFFs) of populations via genotyping by sequencing. *PLoS One*, *8*, e57438. https://doi.org/10.1371/journal.pone.0057438

Cashman, P. A. (2014). *Differential productivity and persistency responses to simulated and animal grazing of perennial ryegrass genotypes*, Belfast: . Queens University Belfast. PhD Thesis. Retrieved from https://www.qub.ac.uk/

Dillon, P. (2006). Achieving high dry-matter intake from pasture with grazing dairy cows. In A. Elgersma, J. Dijkstra, & S. Tamminga (Eds.), *Fresh herbage for dairy cattle* chapter, 1 (pp. 1–26). The Netherlands: Springer.

Elshire, R. G., Glaubits, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, *6*(1–10), e19379. https://doi.org/10.1371/journal.pone.0019379

Emile, J. E., Génier, G., & Guy, P. (1993). Valorisation par des vaches laitières de 2 génotypes de luzerne. *Fourrages*, *134*, 255–258.

Flores-Lesama, M., Hazard, L., Betin, M., & Emile, J.-C. (2006). Differences in sward structure of ryegrass cultivars and impact on milk production of grazing dairy cows. *Animal Research*, *55*, 25–36. https://doi.org/10.1051/animres:2005044

Gauch, H. G. (1992). *Statistical analysis of regional yield trials: AMMI analysis of factorial designs* (1st ed.). Amsterdam, The Netherlands: Elsevier. (Chinese ed., China National Rice Research Inst., Hangzhou, China, 2001.). 278 pp. Retrieved from https://www.elsevier.com/books/statistical-analysis-of-regional-yield-trials-ammi-analysis-of-factorial-designs/gauch-jr/978-0-444-89240-9

Gerber, P. J., Hristov, A. N., Henderson, B., Makkar, H., Oh, J., Lee, C., … Oosting, S. (2013). Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: A review. *Animal*, *7*(2), 220–234. https://doi.org/10.1017/S1751731113000876

Ghosh, P. K., & Mahanta, S. K. (2014). Carbon sequestration in grassland systems. *Range Management & Agroforestry*, *35*(2), 173–181. ISSN 0971–2070.

Gillet, M., & Jadas-Hécart, J. (1965). La flexibilité des feuilles, critère de sélection de la fétuque élevée en tant que facteur d'appétibilité. *Fourrages*, *22*, 6–11.

Gilliland, T. J., & Gensollen, V. (2010). Review of the protocols used for assessment of DUS and VCU in Europe-perspectives. In C. Huyghe (Ed.), *Sustainable use of genetic diversity in forage and turf breeding* (pp. 261–275). Dordrecht, The Netherlands: Springer.

Gilliland, T. J., Hennessy, D., & Ball, T. (2018). Breeding resilient cultivars for European grass based ruminant production systems. *Grassland Science in Europe*, *23*, 29–42.

Hanrahan, L., McHugh, N., Hennessy, T., Moran, B., Kearney, R., Wallace, M., & Shalloo, L. (2018). Factors associated with profitability pasture based systems of milk production. *Journal of Dairy Science*, *101*, 5474–5485. https://doi.org/10.3168/jds.2017-13223

Hynes, D. N., Stergidias, S., Gordon, A. W., & Yan, T. (2016). Effects of concentrate crude protein contents on nutrient digestibility, energy utilization and methane emissions of lactating dairy cows fed fresh-cut perennial grass. *Journal of Dairy Science*, *99*(11), 8858–8866.

INRA, Institut National de la Recherche Agronomique. (2018). *Alimentation des ruminants: Apports nutritionnels, besoins et réponses des animaux, rationnement, tables de valeur- des aliments*, 4ème édition (p. 728). Versailles Cedex, France: Editions Quae. https://www.quae.com/produit/1523/9782759228683/alimentation-des-ruminants

ISF. (2009). *International seed federation, guidelines for handling a dispute on essential derivation in ryegrass*. Retrieved from www.worldseed.org/wp-content/uploads/2015/10/Guidelines_EDV_Ryegrass_Nov_2009.pdf (November 2019).

Jongman, R. H. G., Bunce, R. G. H., Metzger, M. J., Mücher, C. A., Howard, D. C., & Mateus, V. L. (2006). Objectives and applications of a statistical environmental stratification of Europe. *Landscape Ecology*, *21*, 409–419. https://doi.org/10.1007/s10980-005-6428-0

Julier, B., Barre, P., Lambroni, P., Delaunay, S., Thomasset, M., Lafaillette, F., & Gensollen, V. (2017). *'Use of GBS for lucerne variety distinctness, Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular'*. La Rochelle, France, November 7 to November 10, 2017. Retrieved from http://www.upov.int/edocs/mdocs/upov/en/bmt_16/bmt_16_17.pdf

Julier, B., Barre, P., Lambroni, P., Delaunay, S., Thomasset, M., Lafaillette, F., & Gensollen, V. (2018). Use of GBS markers to distinguish among lucerne varieties, with comparison to morphological traits. *Molecular Breeding*, *38*, 133. https://doi.org/10.1007/s11032-018-0891-1

Julier, B., Guines, F., Ecalle, C., & Huyghe, C. (2001). From description to explanation of variations in alfalfa digestibility. In I. Delgado, & I. Lloveras (Eds.), *Qualité de la luzerne et des medics pour la production animale. Options Méditerranéennes. Série A : Séminaires Méditerranéens* (pp. 19–23). Paris, France: CIHEAM.

Julier, B., Huyghe, C., & Ecalle, C. (2000). The within-cultivar part of genetic variance is higher for morphological traits and yield than for digestibility and fiber content in alfalfa. *Crop Science*, *40*, 365–369.

Lila, M. (1977). Influence des modalités de séchage sur la mesure de la teneur des fourrages en éléments azotés et glucidiques. Conséquences lors des récoltes d'essais en champs. *Annales De L'amélioration Des Plantes*, *27*, 465–475.

Liu, X. P., & Yu, L. X. (2017). Genome-Wide Association Mapping of Loci Associated with Plant Growth and Forage Production under Salt Stress in Alfalfa (Medicago sativa L.). *Frontiers in Plant Science*, *8*, 1–13. https://doi.org/10.3389/fpls.2017.00853

Maamouri, A., Louarn, G., Béguier, V., & Julier, B. (2017). Performance of lucerne genotypes for biomass production and nitrogen content differs in monoculture and in mixture with grasses and is partly predicted from traits recorded on isolated plants. *Crop & Pasture Science*, *68*, 942–951. https://doi.org/10.1071/CP17052

Masson, F., & Leclerc, C. (2014). Le Catalogue Officiel : Un outil évolutif au service de l'agriculture et de sa multiperformance. *Agronomie, Environnement & Sociétés*, *4*, 37–45.

McDonagh, J., O'Donovan, M., McEvoy, M., & Gilliland, T. J. (2016). Genetic gain in perennial ryegrass (*Lolium perenne*) varieties 1973 to 2013. *Euphytica*, *212*, 187–199. https://doi.org/10.1007/s10681-016-1754-7

Meehan, E. J., & Gilliland, T. J. (2019). Differences in dry matter content between forage varieties of Lolium perenne L. *Biology and Environment: Proceedings of the Royal Irish Academy*, *119B*(2-3), 123–137. https://doi.org/10.3318/bioe.2019.11

Merry, R. J., Lee, M. R. F., Davies, D. R., Dewhurst, R. J., Moorby, J. M., Scollan, N. D., & Theodorou, M. K. (2006). Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. I. In vitro and in vivo studies of nitrogen utilization. *Journal of Animal Science*, *84*, 3049–3060. https://doi.org/10.2527/jas.2005-735

Metzger, M. J., Bunce, R. G. H., Jongman, R. H. G., Mücher, C. A., & Watkins, J. W. (2005). A climatic stratification of the environment of Europe. *Global Ecology & Biogeography*, *14*, 549–563. https://doi.org/10.1111/j.1466-822X.2005.00190.x

Miller, L. A., Baker, D. H., Theodorou, M. K., MacRae, J. C., Humphreys, M. O., Scollan, N. D., & Moorby, J. M. (2001). Efficiency of nitrogen use in dairy cows grazing ryegrass with different water soluble carbohydrate concentrations. In *Grassland Ecosystems: An Outlook into the 21st Century. Proceedings of the International Grasslands Congress*. (pp. 377–378). Sao Paulo, Brazil. Retrieved from https://www.internationalgrasslands.org/publications

Negussie, E., de Haas, Y., Dehareng, F. R., Dewhurst, J., Dijkstra, J., Gengler, N., … Biscarini, F. (2017). Large-scale indirect measurements for enteric methane emissions in dairy cattle: A review of proxies and their potential for use in management and breeding decisions. *Journal of Dairy Science*, *100*, 2433–2453. https://doi.org/10.3168/jds.2016-12030

Niderkorn, V., & Baumont, R. (2009). Associative effects between forages on feed intake and digestion in ruminants. *Animal*, *3*, 951–960. https://doi.org/10.1017/S1751731109004261

O'Donovan, M., & Delaby, L. (2005). Comparison of perennial ryegrass cultivars differing in heading date, grass ploidy with spring calving dairy cows grazed at two different stocking rates. *Animal Research*, *54*(5), 337–350. https://doi.org/10.1051/animres:2005027

O'Donovan, M., McHugh, N., McEvoy, M., Grogan, D., & Shalloo, L. (2016). Combining seasonal yield, silage dry matter yield, quality and persistency in an economic index to assist perennial ryegrass variety selection. *Journal of Agricultural Science*, *155*(4), 56–568. https://doi.org/10.1017/S0021859616000587

Pembleton, L. W., Drayton, M. C., Bain, M., Baillie, R. C., Inch, C., Spangenberg, G. C., … Cogan, N. O. I. (2016). Targeted genotyping-by-sequencing permits cost-effective identification and discrimination of pasture grass species and cultivars. *Theoretical and Applied Genetics*, *129*, 991–1005. https://doi.org/10.1007/s00122-016-2678-2

Perren, R. (1995). The great agricultural depression of 1879-1896. *Agriculture in Depression, 1870–1940*. , New Studies in Economic and Social History, (p. 7–16). Cambridge, UK: Cambridge University Press. ISBN: 9780521552851.

Peyraud, J. L., & Astigarraga, L. (1998). Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: Consequences on animal nutrition and N balance. *Animal Feed Science and Technology*, *72*(3), 235–259. https://doi.org/10.1016/S0377-8401(97)00191-0

RIIA. (2019). Meat analogues, considerations for the EU. In A. Froggatt & L. Wellesley (Eds.). Chatham House, the Royal Institute of International Affairs. pp 44. ISBN 978 1 78413 312 2. Retrieved from https://www.chathamhouse.org/sites/default/files/2019-02-18MeatAnalogues3.pdf

Roldan-Ruiz, I., Calsyn, E., Gilliland, T. J., Coll, R., Van Eijk, M. J. T., & De Loose, M. (2000). Estimating Genetic conformity between related ryegrass (Lolium) varieties, II. Aflp-based markers. *Molecular Breeding*, *6*, 593–602. https://doi.org/10.1023/A:1011398124933

Roldan-Ruiz, I., van Eeuwijk, F. A., Gilliland, T. J., Dubreuil, P., Dillmann, C., Lallemand, J., … Baril, C. P. (2001). A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (Lolium perenne L.) varieties. *Theoretical and Applied Genetics*, *103*, 1138–1150. https://doi.org/10.1007/s001220100571

Sampoux, J. P., Baudouin, P., Bayle, B., Béguier, V., Bourdon, P., Chosson, J. F., … Viguié, A. (2011). Breeding perennial grasses for forage usage: An experimental assessment of trait changes in diploid perennial ryegrass (*Lolium perenne* L.) cultivars released in the last four decades. *Field Crops Research*, *123*, 117–129. https://doi.org/10.1016/j.fcr.2011.05.007

Sampoux, J. P., Giraud, H., & Litrico, I. (2020). Which recurrent selection scheme to improve mixtures of crop species? *Theoretical Expectations, G3*(10), 89–107. https://doi.org/10.1534/g3.119.400809

Silvey, V. (1978). Methods of analysing NIAB variety trials over many sites and several seasons. *Journal of the National Institute of Agricultural Botany*, *14*, 385–400.

Springmann, M., Clark, M., Mason-D'Croz, D., Wiebe, K., Bodirsky, B. L., Lassaletta, L., … Willett, W. (2018). Options for keeping the food system within environmental limits. *Nature*, *562*, 519–525. https://doi.org/10.1038/s41586-018-0594-0

Tabel, C., & Allerit, R. (2005). Bilan du progrès génétique obtenu pour différents caractères et différentes espèces. *Fourrages*, *183*, 365–376.

Theodoridou, K., Aufrere, J., Andueza, D., Pourrat, J., Le Morvan, A., Stringano, E., … Baumont, R. (2010). Effects of condensed tannins in fresh sainfoin (Onobrychis viciifolia) on in vivo and in situ digestion in sheep. *Animal Feed Science and Technology*, *160*, 23–38. https://doi.org/10.1016/j.anifeedsci.2010.06.007

Tubritt, T., Gilliland, T. J., Delaby, L., & O'Donovan, M. (2018). Comparison of grass utilisation performance of perennial ryegrass varieties. *Grassland Science in Europe*, *22*, 54–56.

van Wart, J., van Bussel, L. G. J., Wolf, J., Licker, R., Grassini, P., Nelson, A., … Cassman, K. G. (2013). Use of agro-climatic zones to upscale simulated crop yield potential. *Field Crops Research*, *143*, 44–55. https://doi.org/10.1016/j.fcr.2012.11.023

Vérité, R., Journet, M., Flechet, J., Lffaivre, R., Marquis, B., & Ollier, A. (1970). Influence de la teneur en eau et de la déshydratation de l'herbe sur sa valeur alimentaire pour les vaches laitières. *Annales De Zootechnie*, *19*(3), 255–268. https://doi.org/10.1051/animres:19700302

Wilkins, P. W., Allen, D. K., & Mytton, L. R. (2001). Differences in the nitrogen use efficiency of perennial ryegrass varieties under simulated rotational grazing and their effects on nitrogen recovery and herbage nitrogen content. Grass and Forage. *Science*, *55*, 69–76. https://doi.org/10.1046/j.1365-2494.2000.00199.x https://doi.

Wilkins, P. W., & Humphreys, M. O. (2003). Progress in breeding perennial forage grasses for temperate agriculture. *Journal of Agricultural Science*, *140*, 129–150. https://doi.org/10.1017/S0021859603003058

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., Jonell, M., Clark, M., Gordon, L. J., Fanzo, J., Hawkes, C., Zurayk, R., Rivera, J. A., de Vries, W., Sibanda, L. M., Afshin, A., Chaudhary, A., Herrero, M., Agustina, R., Branca, F., Lartey, A., Fan, S., Crona, B., Fox, E., Bignet, V., Troell, M., Lindahl, T., Singh, S., Cornell, S. E., Reddy, K. S., Narain, S., Nishtar, S., & Murray, C. J. L. (2019). Human nutrition requirements. *The Lancet*, *393*(10170), 447–492. https://doi.org/10.1016/S0140-6736(18)31788-4

Wims, C. M., McEvoy, M., Delaby, L., Boland, T. M., & O'Donovan, M. (2013). Effect of perennial ryegrass (*Lolium perenne* L.) cultivars on milk yield of grazing dairy cows. *Animal*, *7*, 410–421. https://doi.org/10.1017/S1751731112001814

Woodfield, D. R., & Easton, H. S. (2004). Advances in pasture plant breeding for animal productivity and health. *New Zealand Veterinary Journal*, *52*(6), 300–310. https://doi.org/10.1080/00480169.2004.36446

Yates, F. (1933). The principles of orthogonality and confounding in replicated experiments. *Journal of Agricultural Science*, *23*, 108–145. https://doi.org/10.1017/S0021859600052916

Yayota, M., Tsukamoto, M., Yamada, Y., & Ohtani, S. (2013). Milk composition and flavor under different feeding systems: A survey of dairy farms. *Journal of Dairy Science*, *96*(8), 5174–5183. https://doi.10.3168/jds.2012-5963

Zhao, Y. G., Annett, R., & Yan, T. (2017). Effects of forage types on digestibility, methane emissions and nitrogen utilization efficiency in two genotypes of hill ewes. *Journal of Animal Science*, *95*, 3762–3771. https://doi.org/10.2527/jas2017.1598

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