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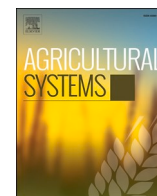
Research Paper

Metabolic assessment of biological mechanisms underlying agroecological systems: The example of parasite dilution and forage niche sharing in mixed-grazing

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HIGHLIGHTS

- Sheep/cattle mixed grazing can improve lamb liveweight gain through forage niche sharing (FNS) and/or parasite dilution (PD).
- We modelled the gains for lambs in terms of metabolizable energy (ME) and crude protein (CP) from PD and FNS.
- Gains in ME from PD were often higher than those from FNS, whereas the opposite was true for CP.
- ME requirements are the most difficult to cover, which suggests a prominent role of PD in sheep/cattle mixed-grazing.
- Using common metabolic metrics helped assess the relative weights of biological mechanisms of contrasting nature.

GRAPHICAL ABSTRACT
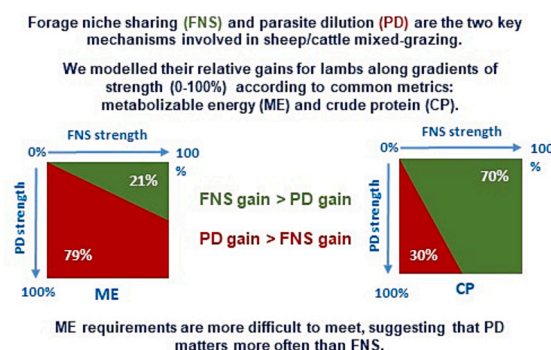
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ABSTRACT

CONTEXT: Mixed-grazing by sheep and cattle is the simultaneous or sequential grazing of a pasture by the two species. It is an agroecological practice known to improve lamb liveweight gains through parasite dilution (PD) and/or forage niche sharing (FNS).
OBJECTIVE: We assessed the combined and relative strengths of the two mechanisms by developing a metabolic approach (French uplands context).
METHODS: We used recently published equations to model the infection cost of gastrointestinal nematodes in metabolizable energy (ME), and crude protein (CP). By comparing infection levels in mixed and monospecific grazing, we quantified the gains of PD in ME and CP. We also used feed value tables to assess the gains in ME and CP resulting from FNS sheep diet improvement. We interpreted these gains in light of ME and CP requirements of lambs. We applied this approach to the dataset of a mixed-grazing experiment, involving sheep monospecific grazing and mixed sheep/cattle grazing, without veterinary treatment. We also applied it to a generic situation where we studied the relative gains in ME and CP, along gradients of increasing strength of PD and FNS.

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RESULTS AND CONCLUSIONS: The approach applied to our experimental data revealed that i) infection by gastrointestinal nematodes can represent 100% of ME and 57% of CP requirements in monospecific grazing, ii) mixed-grazing can reduce these costs to 23% and 12%, respectively and iii) PD was more important than FNS in terms of ME gains, whereas the opposite was true for CP. However, meeting CP requirements was less constraining than meeting ME requirements on the studied grassland type, which puts into perspective the importance of CP gains. With the generic approach, most of the modelled situations also identified PD as the main mechanisms of ME gain (79%), whereas it was FNS for CP (70%), with the same observation that CP requirements were less difficult to meet. Both approaches suggest that in our modelled context, PD matters more often than FNS in mixed-grazing, due to the greater difficulty in meeting ME requirements.

SIGNIFICANCE: We proposed a novel approach to assess the roles of two biological mechanisms of contrasting nature, with the help of common metrics. This approach made it possible to evaluate their combined and relative strength. It has the potential to improve our understanding of the impact of interacting biological mechanisms involved in agroecological grazing systems, and identify prominent ones.

1. Introduction

Agroecology offers a promising pathway to produce food in a sustainable way by optimizing biological processes and reducing chemical inputs (Altieri, 1989). In livestock farming systems, agroecology promotes the use of a diversity of animals, resources and practices (Dumont et al., 2013), among which includes multi-species livestock farming (Martin et al., 2020). Mixed-grazing by sheep and cattle is an example of a multi-species practice that consists of grazing the two species simultaneously, or sequentially, on a given pasture. This type of grazing has been studied for almost half a century (Arundel and Hamilton, 1975; Connolly and Nolan, 1976; Prache et al., 2023), and a literature review by d'Alexis et al. (2014) showed that it can improve lamb growth. The review did not find significant advantages for cattle, even though some studies found marginal growth improvements (e.g. Jerrentrup et al., 2020; Prache et al., 2023).

The improvement of lamb growth can operate through two mechanisms: forage niche sharing and parasite dilution (d'Alexis et al., 2014). Forage niche sharing (FNS) is the mechanism by which species focus on specific sward components. Sheep have a strong preference for legumes and most forb species, while cattle consume a higher proportion of grasses (Walker, 1994; Dumont et al., 2011). By expressing this preference, sheep can select the most nutritive sward components, which can explain their improved performances. In addition, cattle can create and maintain patches of young and nutritive vegetation (du Toit and Olff, 2014), that sheep can graze on after cattle. This facilitates the selection of a forage of good quality which can also improve lamb growth. Parasite dilution (PD) can be defined as the reduction in infection risk that occurs when two species sensitive to distinct pathogens share the same environment (Keesing et al., 2006; Strauss et al., 2015). For example, in ruminants, some of the most important gastrointestinal nematodes are largely host specific, with limited risks of cross-infection in non-host species (e.g. *Haemonchus contortus, Teladorsagia circumcincta* and *Trichostrongylus colubriformis* for sheep; and *Ostertagia ostertagi* and *Cooperia oncophora* for cattle) (Hoste et al., 2003; Rocha et al., 2008). Because of these limited risks of cross-infection, sheep can have lower levels of parasitic gastrointestinal nematodes in sheep/cattle mixed-grazing, compared with sheep monospecific grazing (Arundel and Hamilton, 1975; Marley et al., 2006; Meisser, 2013).

There is both theoretical and practical interest in assessing the combined and relative contributions of PD and FNS to improved lamb growth. Assessing their combined contribution can help us understand how mechanisms add up, and assessing their relative contributions is of interest in terms of monitoring. If a farmer implements mixed-grazing and wants to ensure that he or she obtains the full benefits of the practice, he or she should know which mechanism should be primarily monitored. If PD is prominent, the farmer may want to primarily monitor the level of gastrointestinal nematodes, through the count of excreted eggs per gram of faeces (EPG), as it is currently done by veterinarian laboratories with quick and inexpensive techniques

(Morgan et al., 2005). Conversely, if FNS is prominent, the farmer may primarily want to monitor the quality of diet through faecal analyses, for example, which can be done on-farm with portable devices (Evangelista et al., 2021).

There is no consensus on the relative contribution of PD and FNS in improved lamb performances, but the few conclusive experiments that studied both mechanisms have suggested a higher weight of PD than FNS (Mahieu and Aumont, 2009; Meisser, 2013; Joly et al., 2022; Prache et al., 2023). Most of the time, these conclusions are based on comparative statistical approaches correlating liveweight gains with proxies of PD and FNS. However, it remains difficult to have a clear assessment because of the lack of consistency in the variables used. Although PD is rather homogeneously assessed through standardized counts of excreted EPG, FNS is assessed through a variety of proxies. These proxies involve a diversity of measurements on sward compartments such as sward biomass (overall or according to individual plant species), cover of plant species, sward height, quality of sward (e.g. protein or energy content), or stem/leave proportions (Fraser et al., 2007; Mahieu and Aumont, 2009; Meisser, 2013; Cuchillo-Hilario et al., 2018; Prache et al., 2023). These proxies offer a limited understanding of foraging mechanisms, as they describe the potential intake rather than the actual intake of grazing animals.

We developed a modelling approach to make a consistent assessment of the combined and relative strength of PD and FNS. We quantified the strength of PD and FNS with common metabolic metrics applied to the two mechanisms. We assessed the metabolic costs of infection by parasitic gastrointestinal nematodes in metabolizable energy (ME) and crude protein (CP), with the help of recently published equations (Méndez-Ortíz et al., 2019). We also assessed the improvement in lamb diet occurring when implementing mixed-grazing, with the help of equations assessing the ME and CP concentrations of ingested forage, available in feeding system tables (INRA, 2018). We applied these equations to quantify the combined and relative strength of PD and FNS, and their contribution to improved lamb performance in mixed-grazing systems. We only considered lambs in this study as cattle performance is generally not significantly improved (d'Alexis et al., 2014), as mentioned above.

2. Materials and methods

2.1. Modelling approach

We applied equations to assess the gains in ME and CP occurring when PD reduces parasite infection, and when FNS improves diet quality (Fig. 1). These gains of PD and FNS were combined to assess the full gains of mixed-grazing, and then compared to assess which mechanism has the highest contribution to improved sheep performance. We performed this comparison for both ME and CP and for example, if the ME gains from PD were higher than those of FNS, we considered that PD had a higher metabolic contribution than FNS to improved sheep

performance in mixed-grazing (for ME). In contrast, if the ME gains from FNS were higher, we considered that FNS had a higher metabolic contribution than PD to improved sheep performance in mixed-grazing (for ME).

We first applied this approach to an experiment conducted in temperate grasslands of *Massif Central*, an uplands area of central France (full details in Joly et al., 2022). We conducted this experiment at the INRAE experimental farm of Herbipôle (doi:10.15454/1.557231805050 9348E12) over the grazing seasons of 2019 and 2020 (mid-May to late October). In this experiment we recorded monthly sheep liveweight, excreted nematode EPGs, faecal nitrogen and diet selection by sheep, grazing alone or with cattle (*Holstein* heifers). During the experiment we did not use any chemical drugs against gastrointestinal nematodes, such that infection levels exceeded the 500 EPGs usually recommended for a treatment (Tarazona, 1986) (especially by the end of the grazing season (Table 1)). We used *Romane* ewe lambs of 5 to 9 month old (age at the beginning of the grazing season) as they were not too shy to allow observation of their feeding behaviour, which we used to assess their diet (younger lambs would have been too easily scared by observers). The sessions of observations took place at three periods of the grazing seasons and helped us assess which pasture niches were consumed (details in 2.2 and 2.3). Observations took place on 5 individuals per replicate and grazing type (mixed or monospecific), chosen for their representativeness in terms of weight and EPG at the beginning of the experiment (same individuals used throughout a given experimental year). Two sessions of four hours took place per period (a first one starting just after sunrise and a second one finishing just before sunset). Overall the experimental design involved 140 ewe lambs and 20 cattle over 2019 and 2020, and feeding behaviour observations took place on 60 ewe lambs. We established through a statistical and comparative approach that on average, over the grazing season, PD was the main mechanism of improved sheep liveweight gain (Joly et al., 2022). We examined here whether we obtain the same conclusion through a metabolic lens.

Second, to avoid fitting too much to the experiment, we applied this approach in a generic way. We scanned gradients of increasing strength of PD and FNS with the help of two-dimensional matrices, defined by the gradients of strength, and containing cells in which we compared PD and FNS gains (further details below). We established the proportions of cells in the matrices in which the PD strength was higher than that of FNS, for both ME and CP. This proportion helped us assess which mechanism was prominent in mixed-grazing.

2.2. Metabolic assessments of FNS and PD gains

In Section 2.2, we present the equations used to conduct our

Table 1
Parameters of the experiment using ewe lambs[1].

System	Egg per gram of faeces (no)	Forage niches in diet (% of mouthfuls)			Live-weight (kg)	Live-weight gain (g/day)[3]
		Young vegetative grass[2]	Mature repro-ductive grass	Legume		
Monospecific grazing						
June	338	99	0	1	46	50
July	533	25	75	0	48	50
September	2818	17	83	0	45	50
Mixed-grazing						
June	255	98	0	2	47	50
July	390	44	55	1	50	50
September	850	27	71	2	52	50

[1] Data for ewe lamb from the experiment fully described in Joly et al. (2022) except liveweight gain from OS Romane (2021).

[2] After June the forage niche 'young vegetative grasses' mostly corresponds to patches of short vegetation created by grazing and maintained in vegetative and nutritive conditions (especially by cattle in mixed-grazing).

[3] Liveweight gain used to estimate usual diet requirements for this class of animal and not to fit observed liveweight.

metabolic assessment. Their application to our experimental dataset and according to the generic approach is presented in Sections 2.3 and 2.4. The equations does not consider interactions between FNS and PD, as none were observed in our experiment (Joly et al., 2022). PD and FNS equations are thus independent.

2.2.1. Gains obtained from FNS

To assess the metabolic gains obtained from FNS, we compared the quality of forage ingested under mixed and monospecific grazing. These gains in ME and CP are denoted G_{ME}^{fns} and G_{CP}^{fns}, respectively (Eqs. (1) and (2)).

$$G_{ME}^{fns} = ME_{mixed}^{ingest} - ME_{mono}^{ingest} \tag{1}$$

$$G_{CP}^{fns} = CP_{mixed}^{ingest} - CP_{mono}^{ingest} \tag{2}$$

where ME_{mixed}^{ingest} and ME_{mono}^{ingest} are the amount of ME ingested by sheep under mixed and monospecific grazing (MJ/day), respectively. CP_{mixed}^{ingest} and CP_{mono}^{ingest} are the amount of CP ingested by sheep under mixed and monospecific grazing (g/day), respectively. We assessed ME_{mixed}^{ingest}, ME_{mono}^{ingest}, CP_{mixed}^{ingest} and CP_{mono}^{ingest} according to the dry matter intake in g/day

Mechanism	Variables		Calculation of gains
	Monospecific sheep system (*reference*)	Mixed sheep/cattle system	
Forage niche sharing	ME_{mono}^{ingest} & CP_{mono}^{ingest}	ME_{mixed}^{ingest} & CP_{mixed}^{ingest}	$ME_{mixed}^{ingest} - ME_{mono}^{ingest}$ $CP_{mixed}^{ingest} - CP_{mono}^{ingest}$
Parasite dilution	ME_{mono}^{infect} & CP_{mono}^{infect}	ME_{mixed}^{infect} & CP_{mixed}^{infect}	$ME_{mono}^{infect} - ME_{mixed}^{infect}$ $CP_{mono}^{infect} - CP_{mixed}^{infect}$

Fig. 1. Calculation of the gains generated by mixed-grazing mechanisms (forage niche sharing and parasite dilution) in metabolizable energy (ME) and crude protein (CP).

ME^{ingest} and CP^{ingest}: daily amount of ME and CP ingested by sheep, respectively, based on feed tables of INRA (2018).

ME^{infect} and CP^{infect}: daily cost of infection by gastrointestinal nematode parasites for sheep in ME and CP, respectively, based on Méndez-Ortíz et al. (2019).

of animals (DMI), and the composition of this intake. DMI was evaluated based on DMI_{LW}^{ref}, the amount of dry matter of an ingested forage of reference in g/day (further explanation below). DMI_{LW}^{ref} was based on INRA (2018) and expressed according to Eq. (3)

$$DMI_{LW}^{ref} = 75 \cdot LW^{0.75} \tag{3}$$

where LW is the liveweight in kg. To assess the composition of DMI, we used the forage typology established during the behaviour observations, conducted during our experiment. This typology uses three niches: i) young vegetative grass, ii) mature reproductive grass and iii) legumes. These forage niches have different ME and CP concentrations, and these concentrations vary along the grazing season (INRA, 2018) (Table 2).

To account for the fact that these niches have different ingestibilities compared with the reference forage, we used the fill unit concept of INRA (2018). This concept helps assess how much of a forage can be consumed by comparison with the reference forage (Jarrige et al., 1986). It uses the fill unit values of forages expressed as the ratio of ingested forage of reference to the ingested studied forage. By definition, the reference forage has a fill unit of one and corresponds to a grass with specific characteristics (e.g. 150 g of CP, 280 g of acid detergent fiber, 530 g of neutral detergent fiber for 1 kg of dry matter (DM); details in INRA, 2018).

Based on this fill unit ratio, we expressed ME_{mixed}^{ingest}, ME_{mono}^{ingest}, CP_{mixed}^{ingest} and CP_{mono}^{ingest} with the help of Eqs. (4) and (5).

$$ME_{gs}^{ingest} = \sum_i DMI_{LW}^{ref} \cdot p_{i,gs} \cdot C_i^{ME} \Big/ (1000 \cdot FU_i) \tag{4}$$

$$CP_{gs}^{ingest} = \sum_i DMI_{LW}^{ref} \cdot p_{i,gs} \cdot C_i^{CP} \Big/ (1000 \cdot FU_i) \tag{5}$$

where gs represents the grazing system defined by its type of grazing (mixed or monospecific), period of grazing, and animal characteristics (liveweight and liveweight gains). $p_{i,gs}$ is the percentage of forage niche of type i in the animal diet (unitless), FU_i is the number of fill units of forage i (unitless), and C_i^{ME} and C_i^{CP} are the concentrations in ME and CP (MJ/kg DM and g/kg DM, respectively).

2.2.2. Gains obtained from PD

To assess the metabolic gains obtained from PD, we applied the same comparative approach as for FNS. We compared the metabolic cost of gastrointestinal infection under mixed and monospecific grazing. The gains in ME and CP are denoted G_{ME}^{pd} and G_{CP}^{pd}, respectively (Eqs. (6) and (7)).

$$G_{ME}^{pd} = ME_{mono}^{infect} - ME_{mixed}^{infect} \tag{6}$$

$$G_{CP}^{pd} = CP_{mono}^{infect} - CP_{mixed}^{infect} \tag{7}$$

where ME_{mono}^{infect} and ME_{mixed}^{infect} are the costs of parasitism in ME under monospecific and mixed grazing (MJ/day), respectively. CP_{mono}^{infect} and CP_{mixed}^{infect} are the costs of parasitism in CP under monospecific and mixed grazing (g/day), respectively. To assess ME_{mono}^{infect}, ME_{mixed}^{infect}, CP_{mono}^{infect} and CP_{mixed}^{infect} we used the equations given by Méndez-Ortíz et al. (2019). These equations quantify the costs in ME and CP of parasitism based on the number of adult gastrointestinal nematodes infecting animals. They estimated the cost of one adult worm at 0.056 kJ of ME/kg $LW^{0.75}$ and 0.30 mg of CP/kg $LW^{0.75}$. We adapted the equations to express ME_{mixed}^{ingest} and ME_{mono}^{ingest} in MJ/day, and CP_{mixed}^{ingest} and CP_{mono}^{ingest} in g/day to have units consistent with FNS gains (Eqs. (8) and (9)).

$$ME_{gs}^{infect} = 5.6 \cdot n_{gs}^{agn} \cdot LW^{0.75} \cdot 10^{-5} \tag{8}$$

$$CP_{gs}^{infect} = 3 \cdot n_{gs}^{agn} \cdot LW^{0.75} \cdot 10^{-4} \tag{9}$$

where n_{gs}^{agn} is the number of adult gastrointestinal nematodes in grazing system gs.

2.2.3. Assessment of animal requirements

The above equations helped us assess the gains from PD and FNS in ME and CP and we interpreted these gains with the help of the ME and CP requirements. This step was necessary as a gain in ME (or CP) mostly matters if animal requirements are difficult to meet. If requirements in ME (or CP) are easily met, a gain in ME (or CP) matters less. We evaluated the metabolic balance of animals according to Eqs. (10) and (11).

$$Bal_{gs}^{ME} = ME_{gs}^{ingest} - ME_{gs}^{req} - ME_{gs}^{infect} \tag{10}$$

$$Bal_{gs}^{CP} = CP_{gs}^{ingest} - CP_{gs}^{req} - CP_{gs}^{infect} \tag{11}$$

where for system gs, Bal_{gs}^{ME}, and Bal_{gs}^{CP}, are the ME and CP balances, and ME_{gs}^{req} and CP_{gs}^{req} are the ME and CP requirements, respectively. We calculated ME_{gs}^{req} and CP_{gs}^{req} based on the system of INRA (2018), which uses LW in kg and LW gains in g/day. The equations used to assess ME_{gs}^{req} and CP_{gs}^{req} are presented in the Supplementary Materials.

2.3. Metabolic assessment of PD and FNS applied to our experiment

To assess the relative contributions of parasite dilution and forage niche sharing in our experiment, we used Eqs. (1) to (11) with the experimental dataset[1]. To fit the conditions of the experiment, gs referred to six different configurations, defined by two types of grazing (monospecific and mixed grazing), and three periods. These periods correspond to: i) spring (June) before flowering of major sward components, (ii) summer (late July) when sward heterogeneity was expected to be maximal and (iii) autumn (September (or early October)) when cumulative grazing treatment effects were expected to be maximal.

To assess the gains from FNS, $p_{i,gs}$ was evaluated from the percentages of mouthfuls according to niches recorded during behaviour observations (details in Joly et al., 2022). The vegetative grass eaten by ewe lambs in July or September mostly corresponds to patches of short grass maintained in a vegetative state by grazing, especially by cattle grazing in mixed-grazing. LW was obtained from the weights of animals monitored during our experiment and the LW gains used to assess requirements were obtained from local guidelines (OS Romane, 2021). We used these guidelines to fit the usual performance for this type of animal (ewe lambs), and avoid overfitting experimental conditions (Table 1). FU_i, C_i^{ME} and C_i^{CP} were obtained from INRA (2018), and their values are presented in Table 2.

To assess gastrointestinal nematode infection we monitored the number of EPGs rather than n_{gs}^{agn}, the number of adult worms in the digestive tract. The number of adults is indeed more difficult to estimate, as it requires killing the experimental sheep (Gaba et al., 2006). We derived n_{gs}^{agn} from Cabaret et al. (1998), which provides a model to estimate adult abundance from EPG (Eq. (12)).

$$n_{gs}^{agn} = 10^{\left(-0.22+1.24 \cdot log\left(n_{gs}^{epg}\right)\right)} \tag{12}$$

where n_{gs}^{epg} is the nematode EPG related to grazing system gs (Table 1).

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[1] We had two mixed-grazing systems in our experiment and here we used the mixed system with the highest percentage of cattle in livestock units in the group (~80%) (called Mixed+ in Joly et al., 2022). We provide this for information only as the gains in the two types of mixed-grazing compared with monospecific grazing were not significantly different.

Table 2
Characteristics of grazed forage[1].

	June			July			September		
	Fill Units of one kg of DM[3]	ME[4] content MJ/kg of DM	CP[5] content g/kg of DM	Fill Units of one kg of DM	ME content MJ/kg of DM	CP content g/kg of DM	Fill Units of one kg of DM	ME content MJ/kg of DM	CP content g/kg of DM
Young vegetative grass	1.12	10.87	149	1.12	10.87	149	1.12	10.87	149
Mature reproductive grass[2]	–	–	–	1.39	8.51	92	1.39	8.51	92
Legume	0.83	12.39	249	0.83	11.94	229	0.83	11.94	229

[1] From INRA (2018).
[2] No mature reproductive grass in June.
[3] Dry matter.
[4] Metabolizable energy.
[5] Crude protein.

2.4. Metabolic assessment of FNS and PD according to the generic approach

2.4.1. Matrix approach of the generic assessment

To assess the relative contributions of PD and FNS in a generic way, we used a matrix approach. We applied this approach to 1/the ewe lambs of our experiment and 2/weaned younger lambs of 30 kg exclusively pasture-fed. We used this type of younger animals that were not used in our experiment because they were too shy to be approached during behaviour observations. However they are more important in terms mutton production through their LW gains than older ewe lambs, that are prominently used for flock renewal.

We built matrices defined by two axes, representing gradients of increasing strength of PD and FNS. We built the widest gradients possible, through archetypal situations at both ends of the gradients. The first end of the gradient corresponds to the basal monospecific situation where PD and FNS are null, and thus operate at 0% of their strength. The second end of the gradient corresponds to a situation where PD or FNS operate at 100% of their strength. The comparison between both ends of the gradients provided the maximum FNS and PD gains.

For PD, the basal situation corresponds to a level of infection of 20,000 adult worms, which is the highest level of infection reported by Méndez-Ortíz et al. (2019) and is consistent with numbers provided by Cabaret et al. (1998). The full strength situation then corresponds to 0 adult worms, indicating that parasitism is reduced to zero. For FNS, the

basal situation corresponds to the diet observed in monospecific configuration in our experiment in July, which is the middle of the grazing season for pasture-fed lambs in our temperate context. In this situation, sheep is the sole grazing species, meaning that FNS is the weakest possible (there is only one species so niche sharing between species is by definition null). The full FNS strength then corresponds to an ideal diet comprising 70% legumes, 30% young grass and 0% mature grass. It is based on the preferred sheep diet observed by Penning et al. (1997) in a grassland sown in order to provide sheep with the most favourable diet possible.

The matrix is divided into cells representing 10% increments of the maximum gains of PD and FNS. These increments expressed in MJ/day for ME and g/day for CP correspond to 10% of the values returned by Eqs. (1) and (2), applied to the archetypal situations at both ends of our gradients (0 and 100% strength). For example, if the maximum gain of PD is 20 MJ/day, its 10% increment is 2 MJ/day (example of maximum values for young lambs in Table 3). In each matrix cell, we compared the strength of PD and FNS by a simple subtraction of gains. We then established the percentage of positive and negative cells in the matrix, to determine the most prominent driver of mixed-grazing.

To interpret the results of our matrix assessment, we evaluated ME and CP requirements, as we did with the experimental approach. For the renewal ewe lambs we used 48 kg of LW (measured weight in July) and 50 g/day of LW gains following OS Romane (2021), as with the previous approach. For the younger lambs we used 30 kg of LW following Prache

Table 3
Calculation of the maximum gains in metabolizable energy (ME) and crude protein (CP), from niche forage sharing (FNS) and parasite dilution (PD) in mixed-grazing (example for young lambs). The archetypal baseline and ideal situations correspond to 0 and 100% strength of FNS and PD, respectively. The differences in ME and CP between baseline and ideal situations provide the maximum possible gains from FNS and PD.

	Baseline monospecific situation: 0% strength of mixed-grazing processes			Ideal mixed-grazing situation: 100% strength of mixed-grazing processes			Maximum gains	
Parasite dilution	**Infection level**	**Infection costs**		**Infection level**	**Infection costs**			
		ME (MJ/day)	CP (g/day)		ME (MJ/day)	CP (g/day)	ME (MJ/day)	CP (g/day)
Adult worms	20,000 (4426 EPG)	14.36	76.91	0 (0 EPG)	0.00	0.00	14.36	76.91
Forage niche sharing	**Diet composition (%)**	**Diet value**		**Diet composition (%)**	**Diet value**			
		ME (MJ/day)	CP (g/day)		ME (MJ/day)	CP (g/day)	ME (MJ/day)	CP (g/day)
Total	100	6.75	79.70	100	12.48	224.05	5.73	144.35
Young vegetative grass	*25*	*2.33*	*31.97*	*30*	*2.80*	*38.37*		
Mature reproductive grass	*75*	*4.41*	*47.72*	*0*	*0.00*	*0.00*		
Legume	*0*	*0.00*	*0.00*	*70*	*9.68*	*185.68*		

et al. (2023) and three levels of LW gains (75, 100, 150 g/day), to have a pertinent range of animal performance. This range is consistent with the equations given by Méndez-Ortíz et al. (2019), and corresponds to common gains in pasture-fed lambs (OS Romane, 2021).

2.4.2. Sensitivity analysis

To evaluate the robustness of our general assessment using matrices, we conducted a sensitivity analysis. The analysis assessed the effects of different levels of maximum strength of PD and FNS. Practically we reduced the maximum PD and FNS gains in five steps, down to one fifth. We made this reduction independently for PD and FNS, and tested this way a total of 25 configurations (five PD gain levels multiplied by five FNS gain levels). We then conducted calculations of the percentage of cells of the matrix where PD provided the most important gains, in these

25 configurations for both ME and CP.

We did not consider it pertinent to increase the maximum strength of PD and FNS in the sensitivity analysis, as we already used archetypal situations in building our gradients, which yielded very high levels of maximum strength of PD and FNS. For PD, we considered in the full strength situation a configuration where parasitism is reduced to zero, which is a rather virtual configuration, as nematodes never completely disappear in pasture-based systems (in particular because of the over-wintering mechanisms of parasites (Gibbs, 1982)). Regarding the basal situation of PD (no parasitism reduction), we also used maximum values of infection of 20,000 adult worms based on Cabaret et al. (1998) and Méndez-Ortíz et al. (2019), who together include a total of ~30 studies. Therefore, we considered that we used maximum infection levels close to those observed in real farming conditions.
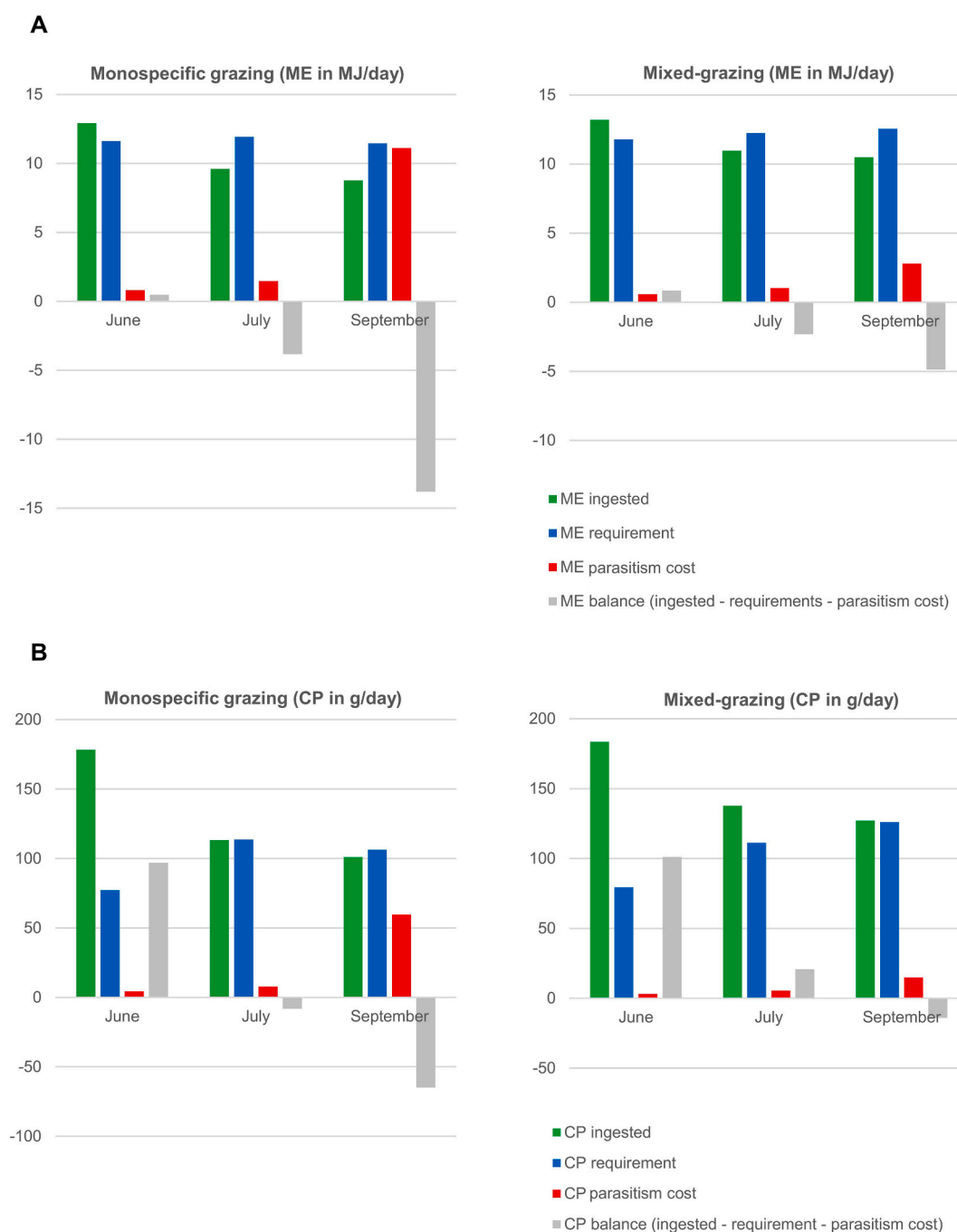

Fig. 2. Metabolic balances in metabolizable energy (ME) (panel A) and crude protein (CP) (panel B) of sheep monospecific grazing and sheep/cattle mixed-grazing.

Regarding FNS, we used a basal situation with no legumes from our actual experimental field configuration of July (see Table 1), and tested a full strength situation where lambs could freely select their diet in sown monocultures of grass and clover, as described by Penning et al. (1997). The lambs were therefore in an ideal situation and virtually ate 70% clover, which is why we did not further increase the amount of legumes. In our maximum-strength situation, all of the grass eaten is young and vegetative, which is the most nutritive stage possible. We therefore think that our FNS full-strength situation cannot be realistically increased.

We conducted the sensitivity analysis on the matrices obtained from the procedures described above. We did it for both ewe lambs and young lambs.

3. Results

3.1. Metabolic assessment in the mixed-grazing experiment

The application of our metabolic assessment to the experimental dataset is summarized in Fig. 2. It indicates that the ME content of the diet (green bar in Fig. 2) is slightly above the requirements in June (blue bar), when vegetation is young and nutritive, for both monospecific and mixed grazing systems. In July and September, when vegetation ages, the ME ingested is slightly below the requirements, with more pronounced differences in monospecific than mixed-grazing. The ME cost of parasitism (red bar) is moderate compared to the ingested ME in June and July, but it becomes high in September, where it represents 100% of the ME requirements in the monospecific system (11 MJ/day), and 23% in the mixed-grazing one (3 MJ/day) (Fig. 2). As a result, the ME balance is negative in July and September for both systems (grey bar), while it should equal zero if ingestion had met requirements and compensated parasitism costs. The ME balance in the monospecific system in September is particularly negative as it corresponds to 127% of ME requirements (−14 MJ/day).

The ingested CP is above the requirements in both systems for most periods, and the CP cost of parasitism is moderate until September. In this latter period the cost represents 57% of CP requirements in the monospecific system (60 g/day), and 12% in the mixed system (15 g/day). As a result, the CP balance is significantly negative (i.e. much below −10% of requirements) only in September in the monospecific system (65 g/day). This deficit under monospecific grazing in September represents 61% of animal CP requirements, whereas it is 127% for ME at the same period and system. Hence, the simulations indicate that ME and CP balances are better in mixed-grazing than in monospecific grazing for all seasons, and that meeting ME requirements is more challenging than meeting CP requirements.

The relative benefits of PD and FNS differ along the grazing season, and are different for ME and CP (Fig. 3). For ME in July, when balances are slightly negative, FNS leads to higher gain than PD. However in September, when balances are strongly negative, the benefits of PD are much higher. For CP, the balance is only strongly negative in September under monospecific grazing, and at that time PD generates higher gains than FNS. When metabolic balances are the most negative, PD thus generates higher gains than FNS, for both ME and CP.

3.2. Generic metabolic assessment

When we applied our metabolic assessment according to the generic matrix approach, we first observed that ME balances are negative, for both ewe lambs and young lambs. For ewe lambs the deficit is − 2.3 MJ/day (−20% of requirements), and for the young lambs the balances are −2.8, −3.2 and − 4.0 MJ/day, for LW gains of 75, 100 and 150 g/day, respectively (−29, −32 and − 37% of requirements). For ewe lambs the CP balance is −0.3 g/day (i.e. −0.27% of requirements) and for young lambs they are +5, −15 and − 63 g/day, for LW gains of 75, 100 and 150 g/day, respectively (+7, −16 and − 44% of requirements). This indicates that for ewe lambs, CP requirements are met but not ME ones. This also indicates that for younger lambs, CP requirements can be adjusted to liveweight gains to avoid deficits, but not ME requirements. This evaluation shows that overall, meeting ME requirements is more difficult than meeting CP requirements, in the simulated context for both sheep age cohorts.

The ME and CP matrices present identical patterns for both animal age cohorts (Fig. 4). It is due to the form of the equations of intake and parasitism that make PD and FNS proportional to LW$^{0.75}$. In the ME matrix, 79% of cells have negative values (Fig. 4), indicating that on the gradients of strength considered, the PD gains are more frequently higher than the FNS gains. Conversely, in the CP matrix, 30% of cells have negative values (Fig. 4), indicating that on the gradients of strength considered, the FNS gains are more frequently higher than the PD gains.

Our sensitivity analysis revealed that the higher gains in ME from PD were confirmed in 88% of the conditions tested (Table S1), and that the higher gains in CP from FNS were confirmed in 76% of the conditions tested (Table S2). Results are identical for both types of sheep. Our sensitivity analysis thus confirms the stability of our generic assessment, especially for the gains in ME from PD.

4. Discussion

4.1. Application of the metabolic assessment to the experimental dataset

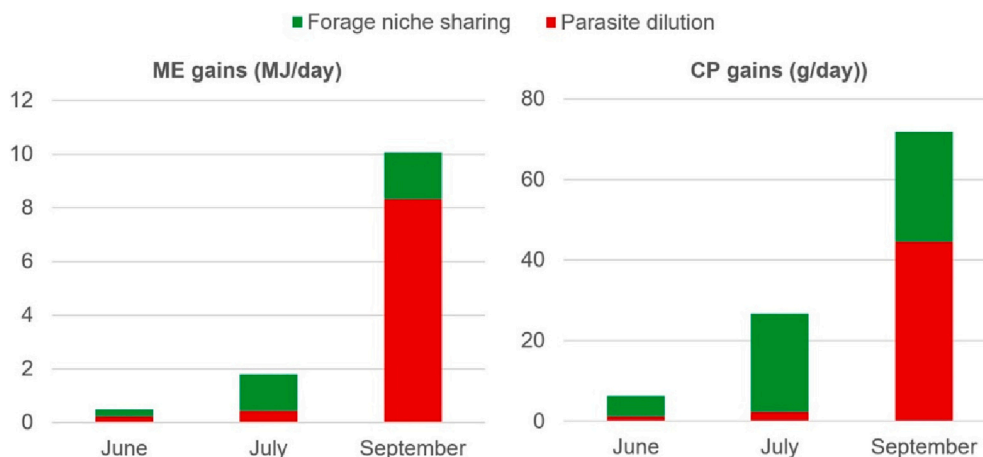The application of the metabolic assessment to our experimental

Fig. 3. Gains in metabolizable energy (ME) and crude protein (CP) from two mechanisms involved in sheep/cattle mixed-grazing (forage niche sharing and parasite dilution).
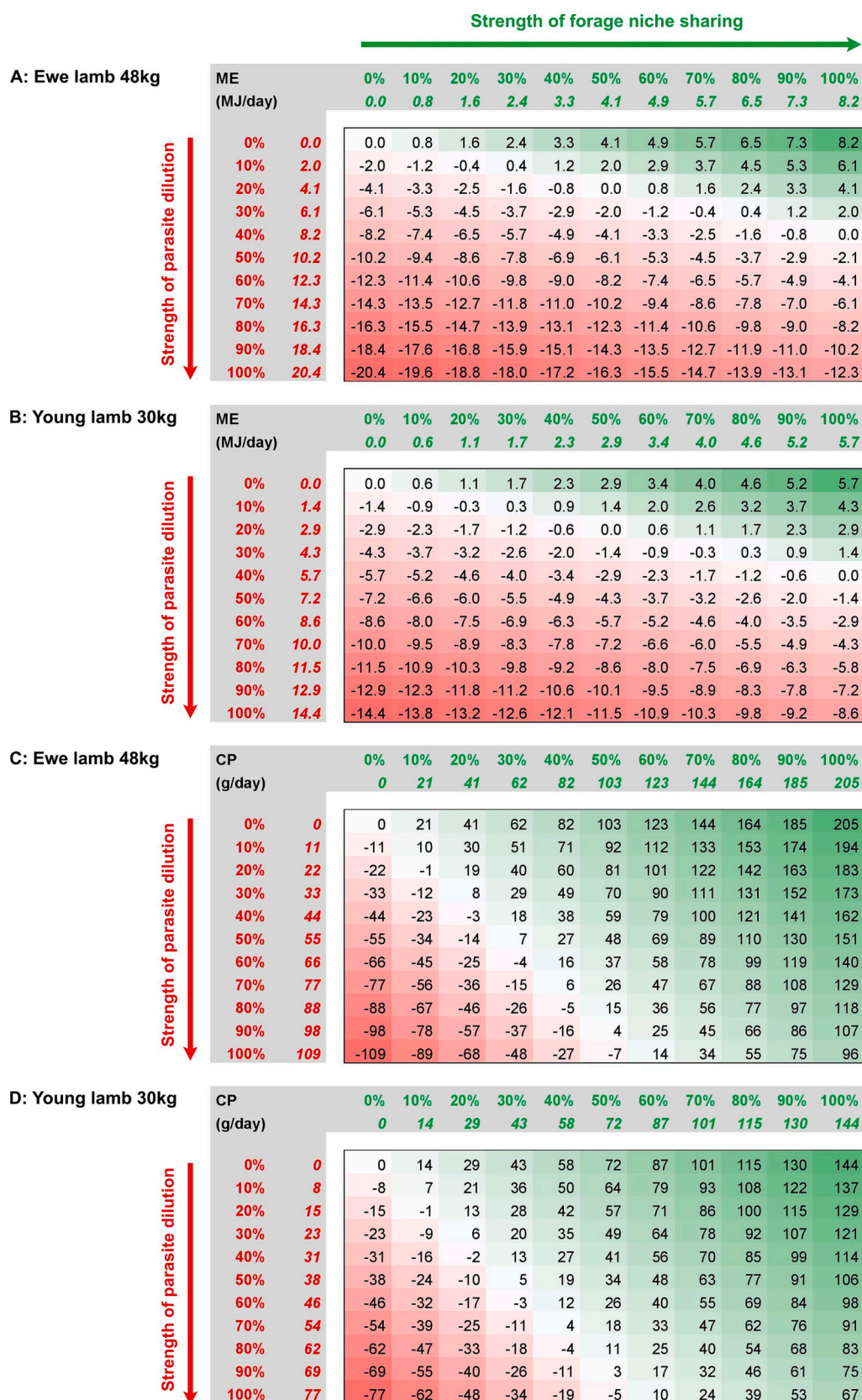
Strength of forage niche sharing →

A: Ewe lamb 48kg

ME (MJ/day)

PD	ME	0% / 0.0	10% / 0.8	20% / 1.6	30% / 2.4	40% / 3.3	50% / 4.1	60% / 4.9	70% / 5.7	80% / 6.5	90% / 7.3	100% / 8.2
0%	0.0	0.0	0.8	1.6	2.4	3.3	4.1	4.9	5.7	6.5	7.3	8.2
10%	2.0	-2.0	-1.2	-0.4	0.4	1.2	2.0	2.9	3.7	4.5	5.3	6.1
20%	4.1	-4.1	-3.3	-2.5	-1.6	-0.8	0.0	0.8	1.6	2.4	3.3	4.1
30%	6.1	-6.1	-5.3	-4.5	-3.7	-2.9	-2.0	-1.2	-0.4	0.4	1.2	2.0
40%	8.2	-8.2	-7.4	-6.5	-5.7	-4.9	-4.1	-3.3	-2.5	-1.6	-0.8	0.0
50%	10.2	-10.2	-9.4	-8.6	-7.8	-6.9	-6.1	-5.3	-4.5	-3.7	-2.9	-2.1
60%	12.3	-12.3	-11.4	-10.6	-9.8	-9.0	-8.2	-7.4	-6.5	-5.7	-4.9	-4.1
70%	14.3	-14.3	-13.5	-12.7	-11.8	-11.0	-10.2	-9.4	-8.6	-7.8	-7.0	-6.1
80%	16.3	-16.3	-15.5	-14.7	-13.9	-13.1	-12.3	-11.4	-10.6	-9.8	-9.0	-8.2
90%	18.4	-18.4	-17.6	-16.8	-15.9	-15.1	-14.3	-13.5	-12.7	-11.9	-11.0	-10.2
100%	20.4	-20.4	-19.6	-18.8	-18.0	-17.2	-16.3	-15.5	-14.7	-13.9	-13.1	-12.3

Strength of parasite dilution ↓

B: Young lamb 30kg

ME (MJ/day)

PD	ME	0% / 0.0	10% / 0.6	20% / 1.1	30% / 1.7	40% / 2.3	50% / 2.9	60% / 3.4	70% / 4.0	80% / 4.6	90% / 5.2	100% / 5.7
0%	0.0	0.0	0.6	1.1	1.7	2.3	2.9	3.4	4.0	4.6	5.2	5.7
10%	1.4	-1.4	-0.9	-0.3	0.3	0.9	1.4	2.0	2.6	3.2	3.7	4.3
20%	2.9	-2.9	-2.3	-1.7	-1.2	-0.6	0.0	0.6	1.1	1.7	2.3	2.9
30%	4.3	-4.3	-3.7	-3.2	-2.6	-2.0	-1.4	-0.9	-0.3	0.3	0.9	1.4
40%	5.7	-5.7	-5.2	-4.6	-4.0	-3.4	-2.9	-2.3	-1.7	-1.2	-0.6	0.0
50%	7.2	-7.2	-6.6	-6.0	-5.5	-4.9	-4.3	-3.7	-3.2	-2.6	-2.0	-1.4
60%	8.6	-8.6	-8.0	-7.5	-6.9	-6.3	-5.7	-5.2	-4.6	-4.0	-3.5	-2.9
70%	10.0	-10.0	-9.5	-8.9	-8.3	-7.8	-7.2	-6.6	-6.0	-5.5	-4.9	-4.3
80%	11.5	-11.5	-10.9	-10.3	-9.8	-9.2	-8.6	-8.0	-7.5	-6.9	-6.3	-5.8
90%	12.9	-12.9	-12.3	-11.8	-11.2	-10.6	-10.1	-9.5	-8.9	-8.3	-7.8	-7.2
100%	14.4	-14.4	-13.8	-13.2	-12.6	-12.1	-11.5	-10.9	-10.3	-9.8	-9.2	-8.6

Strength of parasite dilution ↓

C: Ewe lamb 48kg

CP (g/day)

PD	CP	0% / 0	10% / 21	20% / 41	30% / 62	40% / 82	50% / 103	60% / 123	70% / 144	80% / 164	90% / 185	100% / 205
0%	0	0	21	41	62	82	103	123	144	164	185	205
10%	11	-11	10	30	51	71	92	112	133	153	174	194
20%	22	-22	-1	19	40	60	81	101	122	142	163	183
30%	33	-33	-12	8	29	49	70	90	111	131	152	173
40%	44	-44	-23	-3	18	38	59	79	100	121	141	162
50%	55	-55	-34	-14	7	27	48	69	89	110	130	151
60%	66	-66	-45	-25	-4	16	37	58	78	99	119	140
70%	77	-77	-56	-36	-15	6	26	47	67	88	108	129
80%	88	-88	-67	-46	-26	-5	15	36	56	77	97	118
90%	98	-98	-78	-57	-37	-16	4	25	45	66	86	107
100%	109	-109	-89	-68	-48	-27	-7	14	34	55	75	96

Strength of parasite dilution ↓

D: Young lamb 30kg

CP (g/day)

PD	CP	0% / 0	10% / 14	20% / 29	30% / 43	40% / 58	50% / 72	60% / 87	70% / 101	80% / 115	90% / 130	100% / 144
0%	0	0	14	29	43	58	72	87	101	115	130	144
10%	8	-8	7	21	36	50	64	79	93	108	122	137
20%	15	-15	-1	13	28	42	57	71	86	100	115	129
30%	23	-23	-9	6	20	35	49	64	78	92	107	121
40%	31	-31	-16	-2	13	27	41	56	70	85	99	114
50%	38	-38	-24	-10	5	19	34	48	63	77	91	106
60%	46	-46	-32	-17	-3	12	26	40	55	69	84	98
70%	54	-54	-39	-25	-11	4	18	33	47	62	76	91
80%	62	-62	-47	-33	-18	-4	11	25	40	54	68	83
90%	69	-69	-55	-40	-26	-11	3	17	32	46	61	75
100%	77	-77	-62	-48	-34	-19	-5	10	24	39	53	67

Strength of parasite dilution ↓

Fig. 4. Generic assessment evaluating the relative strengths of forage niche sharing (FNS) and parasite dilution (PD), in metabolizable energy (ME) (Panel A and B) and crude protein (CP) (Panel C and D). The strength of PD and FNS are given in both percentages and ME and CP per day (values in italics next to the percentages). Values in the matrix cells are the differences between the gains in PD and those of FNS, in ME and CP. Negative values (in red) indicate a higher gain from PD than FNS and positive values (in green) indicate a higher gain from FNS than PD.

dataset helps explain the sheep growth patterns observed in mono-specific and mixed grazing; and assess the relative strength of PD and FNS. We first observe that the cost of parasitism appears to be very high compared to animal requirements. Under monospecific sheep grazing, this cost represents 100% of the ME requirements and 57% of the CP requirements, in the late grazing season (September). It thus doubles the requirements of ME and importantly increases those of CP. As a result, metabolic requirements are not met and balances are extremely unfavourable, which can explain the liveweight losses at the end of the grazing season in the monospecific grazing system we observed in our experiment (Joly et al., 2022).

A second finding of the experimental assessment is that mixed-grazing significantly reduces the costs of parasitism. In September, when parasitism is important, they are reduced from 100 to 23% of daily requirements for ME, and from 57 to 12% of daily requirements for CP. It makes it possible to reach much more favourable metabolic balances under mixed-grazing than under monospecific grazing. These metabolic gains are consistent with the observed LW gains, which were much more higher in mixed-grazing than in monospecific grazing (~40 g/day higher) (Joly et al., 2022).

A third finding concerns the gains that can be obtained from PD and FNS, and what they represent when metabolic balances are negative. Our assessment showed that in July, when the ME balance is moderately unfavourable, most of the metabolic gain of mixed-grazing is obtained from FNS. In contrast, in September, when both ME and CP balances are extremely unfavourable, especially for ME, the most important mechanism of mixed-grazing is PD. This result suggests that when metabolic requirements are challenging to meet, PD is the most important mechanism. This finding is consistent with the empirical statistical assessment made in our experiment, which attributed most of the improvement of LW gain to PD (Joly et al., 2022). This result is also consistent with the high parasitism cost in September, as a significant decrease of an important cost mechanically results in a significant gain.

We thus obtained similar results in two different ways: i) an experimental comparative approach to explain LW gain differences, and ii) a modelling approach based on metabolic balances that are likely to impact these LW gains. These two ways are different in their data nature, as the final LW was directly measured in the experiment, while the metabolic balances were modelled. These two different sources of information and approaches are consistent, as they both attributed more weight to PD than to FNS, to explain the improved sheep performances under mixed-grazing.

4.2. Generic metabolic assessment of the relative strength of parasite dilution and forage niche sharing

The general assessment based on a matrix approach provided similar results. The ME balance was negative for all of the LW gains considered and sheep types, whereas the CP balance was modulated by the levels of LW gain for young lambs and neutral for ewe lambs (requirements met). Meeting ME requirements is thus more challenging than meeting CP requirements, in our modelled conditions. The general assessment also showed that the CP gains were mostly obtained through FNS, whereas the ME gains were mostly obtained through PD. Our sensitivity analysis finally showed that these results are robust to changes in maximum gains of FNS and PD, regardless of the sheep age cohort.

From the perspectives of CP and ME balances and the gains obtained through FNS and PD, PD plays a more important role than FNS in mixed-grazing mechanisms. This assessment stresses the importance of PD in improving sheep LW gain in mixed-grazing, which is consistent with the assessment based on our experimental data. Hence, both our experiment and generic approaches suggest that under mixed-grazing, in our context, PD is more often the prominent mechanism involved in improved LW gains. These two types of metabolic assessments are in addition in line with other experiments that studied the relative contributions of PD and FNS (Mahieu and Aumont, 2009; Meisser, 2013;

Prache et al., 2023).

4.3. Metabolic assessment of agroecological practices

We are not aware of previous attempts to use a metabolic approach to assess the benefits of an agroecological practice, in livestock production. This metabolic approach helped integrate two biological mechanisms of contrasting nature (FNS and PD) by using common metrics (ME in MJ/day and CP in g/day). This integration through a common 'currency' made it possible to assess their combined and relative contribution in mixed-grazing, and confirmed the previous results of the experiment of Joly et al. (2022). This experiment applied a typical empirical approach to correlate liveweight gain to parasitism statistically, while here we developed a more functional and causative approach, as the metabolic balance is directly connected to animal growth performance (INRA, 2018). This metabolic approach thus provides an additional level of understanding compared with a statistical and correlative approach. Stressing the importance of PD compared with FNS also helps prioritize biological mechanisms of interest, in the agroecological context of mixed-grazing.

This causative and metabolic approach can improve our understanding of the mechanisms involved in a variety of agroecological systems and grazing management regimes. For example, there is a growing interest in using bioactive plants such as chicory to control gastrointestinal parasites, but this plant only becomes efficient when it represents a high diet share (Peña-Espinoza et al., 2018). It can thus be useful to assess what is saved in terms of parasitic costs with this plant, and what is lost if it replaces a more nutritive forage. Without a common metric, a consistent balance cannot be estimated but it becomes possible with the metabolic variables we used (ME and CP). Using these metrics to make an overall metabolic assessment can thus help define integrated health and feeding strategies in grazing systems.

4.4. Limits and perspectives

Our metabolic assessment is based on recently published equations of metabolic costs, based on the total number of gastrointestinal adult worms in the digestive tract. These equations do not account for the nematode species involved, even though worm communities can be diverse (Cabaret et al., 2002), and species impact different areas of the gastrointestinal tract (Méndez-Ortíz et al., 2019). The relative abundance of species could possibly affect their metabolic impacts, and thus the expected benefits of PD. However, we think that our results pointing towards the prominent role of PD remain valid owing to i) the consistency between the metabolic assessment using the experiment data and the experiment observations (on ewe lambs), ii) the consistency between the metabolic assessments using experiment data and matrices iii) the matrix approach applied to two types of sheep that scanned wide gradients of PD and FNS, and which was backed by sensitivity analysis that scanned different ranges of gradients (we made scans of scans). However, if new equations accounting for the relative abundance of nematode species were published, it would be pertinent to use them to refine our analysis. It would also be interesting to integrate other types of gastrointestinal parasites that can reduce lamb performance, such as coccidia or cestoda, if related equations were published.

The sensitivity to parasitism according to the age of sheep is in addition not taken into account directly in the equations of Méndez-Ortíz et al. (2019). It is present indirectly through the term $LW^{0.75}$ that increases the metabolic cost of parasitism by 1.68 ($2^{0.75}$), when the adult worm load doubles, which can occur when LW doubles while animal grows. However, if new models taking directly into account the age of sheep were available, it would be interesting to use them to refine our results.

In addition, the typology of forage niches we used could be improved. We merged grasses under a single category but several grass species are present in grasslands, and they can have different nutritive

values (INRA, 2018). Additionally, sheep are capable of efficiently sorting the forage components they consume (Garcia et al., 2003; Dumont et al., 2011), and those with the best nutritive value could be selected. So refining the modelled diets from observations with more precise data could further improve assessments. Such data could come from experiments involving chemical markers (Chen et al., 1999) or DNA bar coding (Pompanon et al., 2012), to precisely characterize diet from faeces samples. These refinements could show that even in monospecific grazing, sheep are able to maintain a diet of good quality because of their selectivity. They could further put into perspective the role of FNS compared with PD in mixed-grazing, and provide further evidence of the prominent role of PD.

Besides that our approach, as any modelling approach, requires a calibration step. In our case this calibration step involves data on grass and legume forage quality, which is by nature time and site specific (INRA, 2018). We scanned gradients of increasing strength of PD and FNS to get around this problem, and thus improve the generalizability of our findings. However, we do not assume that our results can be applied to any biogeographic conditions. For example, we used data from temperate oceanic uplands, and thus do not pretend that our conclusions could be extrapolated to boreal or Mediterranean conditions. In addition, the higher contribution of PD could be different in pasture contexts where CP requirements are more difficult to meet than ME requirements.

Finally, we think that our matrix approach involving gradients is an interesting tool to study the use of biological processes and semi-natural resources, as promoted by agroecology (Altieri, 1989; Dumont et al., 2013; Wezel et al., 2014). Biological processes such as PD could have a lower range of efficiency than conventional veterinary treatments based on chemical drugs. Similarly, natural forages can have wider ranges of nutritive values and productivities than standardized and sown varieties of distinct plant species. It is therefore useful to have tools and approaches at disposal to handle heterogeneity and variability. Ultimately such tools could help distinguish solutions that can be generalized along gradients without losing their efficiency, i.e. 'one-size-fits-all solutions' (Dumont et al., 2022), from those that can only be effective within a specific range of ecological, temporal or climatic conditions. This genericity could contribute to the ergonomics and operability of agro-ecological solutions, which is a necessary condition to contribute to their adoption (Joly et al., 2021).

Ethics approval

Inapplicable.

Financial support statement

CRediT authorship contribution statement

Frédéric Joly: Conceptualization, Methodology, Formal analysis, Visualization, Writing – original draft. **Pierre Nozière:** Methodology, Formal analysis, Writing – review & editing. **Philippe Jacquiet:** Methodology, Writing – review & editing. **Sophie Prache:** Methodology, Writing – review & editing. **Bertrand Dumont:** Methodology, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data regarding the experiment used in this study are available from the INRAE repository https://doi.org/10.15454/S7P0HG.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.agsy.2023.103707.

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