Animal 17 (2023) 101030

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Multiphase diets may improve feed efficiency in fattening crossbreed Holstein bulls: a retrospective simulation of the economic and environmental impact

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A R T I C L E I N F O

Article history:
Received 2 March 2023
Revised 1 November 2023
Accepted 2 November 2023
Available online 10 November 2023

Keywords:
Machine-learning
Nutritional requirements
Optimisation
Precision nutrition
Ruminant

A B S T R A C T

Beef industry needs alternative feeding strategies to enhance both economic and environmental sustainability. Among these strategies, adjusting the diet dynamically according to the change of nutritional requirements (multiphase diet) has demonstrated its economic and environmental benefits in pig production systems. Therefore, this retrospective study aims to assess, through simulation, the theoretical economic and environmental benefits of introducing a multiphase diet for crossbreed bulls feeding (one or more diet changes). For this, individual data of BW, BW gain, and daily intake were recorded from 342 bulls during the last fattening period (112 days). These data were used to estimate individual trajectory of energy and protein requirements, which were subsequently divided by individual intake to calculate the required dietary energy and protein concentrations. The area between two functions (i.e., $f1$: constant protein concentration in the original diet during fattening and $f2$: estimated protein concentration requirements) was minimised to identify the optimal moments to adjust the dietary concentration of energy and protein. The results indicated that both energy and protein intake exceeded requirements on average (+16% and +28% respectively, $P < 0.001$), justifying the adoption of a multiphase diet. Modelling the individual trajectories of required metabolisable protein (**MP**, g/kg DM) during the fattening period resulted in exponential decay model in relation to BW [$32120 \times \exp(-0.026 \times \text{BW}) + 59.9$], while the dietary net energy concentration followed a slightly quadratic model [$2.26–0.0026 \times \text{BW} + 0.000003 \times \text{BW}^2$]. Minimisation of the area between curves showed two optimal moments to adjust the diet: at 312 kg and 385 kg of BW, indicating three diet phases: (a) <312 kg, (b) 312–385 kg, and (c) 385–600 kg. For the second and third phases, the dietary energy and protein concentration should be 70 g MP/kg DM and 1.70 Mcal/kg DM and 61 g MP/kg DM and 1.65 Mcal/kg DM, respectively. These diet adjustments might improve economic profitability by 29 €/animal, reduce estimated nitrogen excretions by 16% ($P < 0.001$), and maintain similar weight gain ($P > 0.16$) compared to the commercial diet. However, the decrease in dietary energy concentration led to increased fibre concentration, which in turn increased the estimated CH_4 emissions of animals with the multiphase diet (+44%, $P < 0.001$). Hence, multiphase diet could theoretically reduce feeding cost and nitrogen excretion from fattening cattle. Further *in vivo* studies should confirm these results and find optimal nutritional strategies to improve economic profitability and environmental impact.

Implications

The present retrospective study aimed to assess the potential of multiphase diet, which already demonstrated its benefits in other species, in improving feed efficiency of beef cattle. Results suggest that implementing a multiphase diet (consisting of three phases) throughout the fattening period of Holstein bulls can improve both economic profitability and environmental impact by reducing nitrogen excretion. This improvement was related to decreased metabolisable protein and net energy concentrations in the diet, which has no adverse effects on BW gain. However, an increase in CH_4 emissions was consequently observed as a result of the increase in dietary fibre.

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https://doi.org/10.1016/j.animal.2023.101030
1751-7311/

Introduction

The correct optimisation of nutrient utilisation by livestock animals is a key factor in improving both economic profitability and environmental impact, particularly in beef cattle where feed efficiency tends to be low (Tolkamp, 2010). Over the past 50 years, research has been focused on this subject, with efforts directed towards improving ingredient processing, diet rationing, or testing feed additives (Connor, 2015). Since the development of technologies for continuous and individualised monitoring of animal performance, precision nutrition techniques have emerged as a potent pathway for improving dietary efficiency in ruminants (González et al., 2018). The multiphase diet is one of the precision nutrition techniques that has been suggested by scientific researchers. It involves adjusting nutrient supply according to evolving requirements in growing pigs (Pomar et al., 2015) or lactating dairy cattle (Barrientos-Blanco et al., 2020). In beef cattle, the change of dietary protein concentration during the finishing period has been investigated, with studies exanimating the transition from 13 to 11.5 or 10% CP in the diet (Cole et al., 2006, Klopfenstein and Erickson, 2002). However, these studies did not provide insights into the optimal timing for implementing diet changes.

Nutritional requirements change dynamically as cattle mature, influenced by genetic, sex, growing period, body composition, and the type of diet consumed (Friggens et al., 2013). Therefore, implementing a multiphase diet is well justified to avoid exceeding the nutritional requirements. The utility and advantages of a multiphase diet have already been demonstrated in pig production, primarily because their rations are based on concentrate feeds, which are generally easy to manage and modify, often with the use of automatic electronic feeder (Andretta et al., 2014; 2016).

To implement the multiphase diet in beef cattle, there is a scientific lack regarding when the different growth phases occur throughout their entire fattening period. In several intensive beef production systems, where animals are commonly fed high-concentrate diets, animals are fed one or two different diets (growing and finishing) during the entire fattening period (Keady et al., 2004; Sampaio et al., 2017). When using two different diets, the switch from growing to finishing diets is determined by management strategies (NRC, 2016) and not to adjust nutrient supply to the requirements. This latter has already been developed in pig production, where the different phases are defined by intervals of BW (kg) or age (d)] (Andretta et al., 2014).

Therefore, the objective of this study was to simulate the impact of a multiphase diet in intensive beef cattle fattening, where calves are typically fed high-concentrate diets. To achieve this, the following steps were taken: (1) modelling individual animal requirements and dietary concentration throughout the fattening period, (2) determining the optimal moments to change dietary energy and/or protein, and (3) evaluating, through retrospective simulation, the potential economic and environmental benefits of a multiphase diet compared to commercial feeding management.

We hypothesise that a multiphase diet could result in cost savings and reduced nitrogen (**N**) excretion due to better-adjusted supply of N in the diet.

Material and methods

The present study is a retrospective analysis (a posteriori simulation) based on observed data. The simulation was performed in seven steps: (1) collection of *in vivo* data, (2) estimation of individual requirements, 3) calculation of dietary concentrations of energy and protein required, (4) identification of optimal change points, (5) reformulation of diets, (6) simulation of individual animal response to the new diets, and (7) estimation of economic and environmental impacts. Fig. 1 illustrates the methodological sequence used in this work:

Step 1: Collection of in vivo data

Data sources

The analysis was conducted using individual data of BW, BW gain, and DM intake (**DMI**) from 342 growing crossbred Holstein bulls which initially had an age of 197 ± 39.5 days and a starting BW of 269 ± 70.9 kg. The crossbreed Holstein bulls were born from Holstein Dams and beef cattle sires and were phenotypically identified as Holsteins. Data were collected during two experiments conducted in two periods (2021–2022) on a commercial farm (Agromont, Montgai, Lleida, Spain). After a four-week adaptation period, animals were fed two commercial and similar pelleted concentrates (Diet A and Diet B, as shown in Table 1) until the end of the fattening period. Individual data of DMI, BW, and average daily gain (**ADG**) data were recorded over 126 days of the fattening period. The animals were managed similarly as in Llonch et al. (2023).

Diets and feed management

Animals were fed two different common fattening diets used in intensive production conditions in Spain. These diets consisted of concentrate, and wheat straw with the following composition (on a DM basis): 59 g/kg of CP, 766 g/kg of neutral detergent fibre, and 67 g/kg of ash. Both diets were provided *ad libitum* and in separate feeders. The formulation of both concentrates was done using Brill® software, following INRA (2018) guidelines (more details are presented in the dietary formulation section). The concentrates were distributed in the form of pellets using electronic feeders. Both concentrates had corn grain as the main ingredient, and they then differed regarding their protein source (soybean vs peas meal with distiller's dried grains with solubles (**DDGs**)). Both concentrates had the same amount of palm oil, urea, and premix (minerals and vitamins). Despite variations in ingredient composition, both concentrates had similar concentrations of net energy (1.88 Mcal/kg DM) and metabolisable protein (88.5 g/kg DM).

To conduct chemical composition of diets, feed samples were collected at each feed manufacturing. The analysis included measurements of DM (method 925.04), ash (method 642.05), CP using the Kjeldahl method (method 988.05), NDF using sodium sulphite and alpha-amylase (Van Soest et al., 1991), and ether extract (**EE**) by Soxhlet with prior acid hydrolysis (method 920.39), as described in Sánchez et al., (2022).

Measures of individual animal performances

Animals were weighed every 14 days on an electronic weighing scale (FX1 model, TEXAS TRADING GmbH, Windach, Germany). Average daily gain was calculated as the difference between two consecutive weights, divided by 14. The concentrate intake was recorded daily and individually through electronic feeders (GEA Surge, Westphalia, Germany). The start and the end of each animal's visit were recorded using an antenna located at the concentrate feeder and a transponder placed in the left ear of each bull, as described in Devant et al. (2012). In addition to the concentrate, straw was offered *ad libitum* in a separated five-space straw feeder (3.60 m length, 1.10 m width, and 0.32 m depth). Based on previous works on similar animals and conditions (Marti et al., 2014; Verdú et al., 2015; Llonch et al., 2023), individual straw intake was estimated as 10% of the concentrate intake. Pens were also equipped with one drinker (0.30 m length, 0.30 m width, 0.18 m depth). Total DMI was calculated as the sum of concentrate intake and the estimated straw intake (each one corrected for its DM concentration). Feed efficiency was addressed using the feed conversion efficiency (**FCE**) index, which was calculated as ADG divided

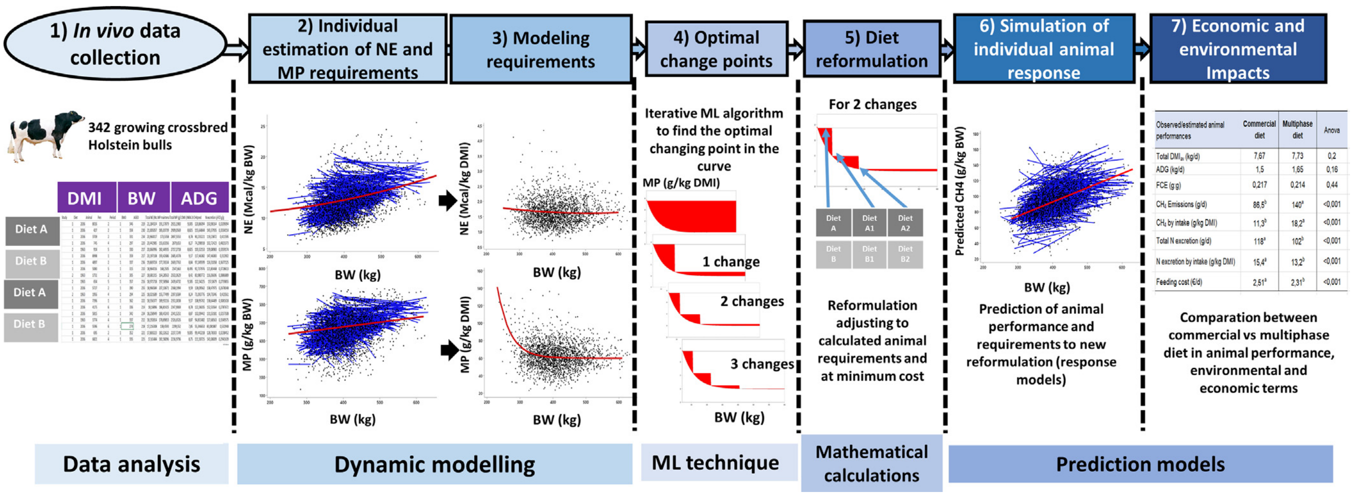

Fig. 1. Methodological steps followed in this work to simulate the impacts of a multiphase vs. commercial diet on crossbred Holstein bulls during fattening period. Abbreviations: DMI = DM intake, ADG = average daily gain, NE = net energy, MP = metabolisable protein, ML = machine learning, FCE = Feed conversion efficiency, CH_4 = enteric methane, N = Nitrogen.

Table 1
Ingredients and chemical composition of the two diets used in crossbred Holstein bulls.

Item	Concentrate A	Concentrate B
Ingredient composition, % inclusion on DM basis		
Corn	33.0	40.0
Barley	15.7	0.00
Zootechnic corn flour	15.0	16.0
Soybean hulls	14.6	9.00
DDGS Corn[1]	14.0	8.00
Wheat middlings	3.50	17.0
Soybean meal	0.00	1.68
Peas meal	4.00	0.00
Palm oil	1.80	1.80
Urea	0.55	0.55
Minerals and vitamin mix[2]	0.20	0.20
Dietary chemical composition (g/kg of DM, or otherwise stated)		
Organic matter	890	890
CP	128	130
NDF	206	194
ADF	103	86.9
Starch	386	398
Starch/NDF (g/g)	1.87	2.05
Net Energy (Mcal/kg DM)	1.89	1.87
MP (g/kg of DM)[3]	88.0	89.0
MP/Net energy (g/Mcal)	46.5	47.5

[1] Distillers Dried Grains with Soluble corn;
[2] Minerals and vitamin mix: 5% P, 25% Ca, 8% Mg, 0.2% Na, vitamin A (30 000 000 IU/kg), vitamin D3 (1 000 000 IU/kg) and vitamin E (30 000 mg/kg).
[3] MP = Metabolisable Protein

by DMI. Finally, to estimate both net energy (**NE**) and metabolisable protein (**MP**) intake, DMI was multiplied by dietary NE and MP concentrations, respectively.

Step 2: Estimation of nutritional requirements and required dietary concentration

Individual requirements of NE (Mcal/day) and MP (g/day) were determined using the INRA guidelines (2018) and the observed performances during the fattening period. Since BW was measured in 14-day periods, the average value of DMI from those 14 days was used. Consequently, animal requirements and dietary concentration were individually estimated for each of these periods. Subsequently, the required dietary energy and protein concentration were calculated as follows:

Dietary NE concentration (Mcal/kg DM) = NE requirements (Mcal/day)/DMI (kg/day).

Dietary MP concentration (g/kg DM) = MP requirements (g/day)/DMI (kg/day).

Step 3: Modelling requirements and dietary concentration across the fattening period

After calculating the individual daily requirements of NE (Mcal/d) and MP (g/d), and the required dietary concentrations of NE (Mcal/kg DMI) and MP (g/kg DMI), these values were modelled as a function of BW for each animal. This modelling allowed for the placement of the optimal dietary changes based on BW. The relationships were modelled using the following mixed effects model:

$$Y_{ij} = \left(B_0 + b_{0,s(d(a))}\right) + \left(B_1 + b_{1,s(d(a))}\right) \times X_{ij} + e_{ij}$$

where Y is the dependent variable (NE requirements (Mcal/d), MP requirements (g/d), dietary NE required (Mcal/kg DMI), or dietary MP required (g/kg DMI)), X is the BW of animals (kg), B_0 and B_1 are the fixed effects (intercept and slope, respectively) and $b_{0,\ s(d(a))}$ and $b_{1,\ s(d(a))}$ are the random effect on the intercept and slope, of the animal ($a = 1\ldots n$ animals) nested within the diet (d = concentrate A or concentrate B), nested within the study (s = study 2019 or study 2020), with $b_{0\ s(d(a))} \sim N(0,\ \sigma^2_{b0})$ and $b_{1,\ s(d(a))} \sim N(0,\ \sigma^2_{b1})$, and $e_{ij} \sim N(0,\ \sigma^2_e)$.

Different structures of random effect were evaluated, from simple (with only the study effect) to nested factors (including the study, diet, and animal). The best random structure was identified from the lowest Akaike Information Criterion (**AIC**), Bayesian Information Criterion (**BIC**), using the restricted maximum likelihood method in the "nlme" library (Pinheiro and Bates, 2006), in R software. In addition, the proportion of the model's variance explained by random and fixed effects was extracted through the r.squaredGLMM function from the "MuMin" library.

In addition to the linear mixed model, quadratic and exponential relationships were also explored, while maintaining the same random structure. The best-fitting model for our data was selected based on the smallest AIC, BIC, and RMSE values. In order to check if the difference in AIC, BIC and RMSE was significant, we conducted an ANOVA. The quadratic or exponential model was considered as best-fitted only if the AIC, BIC and RMSE were smaller than those of the linear model and the le P-value was <0.05.

Step 4: Determining the main change points in dietary concentration across the fattening period

Once the best relationship between BW and the required dietary NE (Mcal/kg DMI) or MP (g/kg DMI) was established, the main change points in terms of dietary energy and protein concentration during the fattening period were identified. In a previous preliminary study (Guarnido-Lopez et al., 2022), the Machine Learning algorithm from library "Changepoint" in R (using "Binseg method") was employed. This function determines significant changing points (Q) across the curves. However, to obtain the change point more accurately, we calculated the area between two curves: (1) the horizontal line of constant dietary concentration (of NE or MP) given by the commercial diet and (2) the model of required NE or MP concentrations. We determined this area using the function "integrate" (Piessens and Branders, 1983) in R Software. Subsequently, using an Machine Learning iterative process, the function "integrate" searched for the point (BW) where proposing a diet change (i.e., reducing NE or MP concentration) would minimise the area between the curves (the horizontal line representing the commercial diet and the model representing our NE and MP requirements). This point in the curve indicated the optimal BW for implementing a diet change by decreasing excess area, which represents excess NE or MP. This iterative process was performed for one, two, and three diet changes.

Step 5: Reformulation of multiphase diets

Experimental concentrates A and B were formulated according to INRA, 2018 guidelines with the aim of maximising growing performances (i.e., ADG) while minimising costs. Once the optimal change points were found, and to simulate the effect of adjusting NE and MP concentrations, diets were reformulated at these points to precisely meet the estimated animal requirements. The reformulation was performed on Brill® software, using the same ingredients as in concentrate A and concentrate B. In this software, the process involves an optimisation of diet cost while meeting the nutritional requirements.

Steps 6 and 7: Estimating animal performance, feeding costs, and environmental impact of multiphase vs. commercial diets

The theoretical animal performance (ADG and feed efficiency) in response to the newly adjusted diet was estimated using INRA, 2018 guidelines (Section II), Hoch et al., (2004), and the body gain composition model for Holstein bulls reported by Diaz et al., (2001). These equations were developed as regression on group data. However, in the present study, individual-specific inputs (animal-specific performances) were incorporated into the equations, making the estimation specific to each animal. Subsequently, the observed and theoretical performances were compared (commercial vs. multiphase diets, respectively). To compare the feed costs between the commercial and the multiphase diets, the average national prices of ingredients were used (https://www.mapa.gob.es/).

Regarding the environmental impact of diets, both total enteric methane (**CH_4**) and N excretion were individually estimated using the following equations:

CH_4 (MJ/d) = $-$ 1.01 + 2.76 $\times$ NDF (kg/d) + 0.722 $\times$ Starch (kg/d); (Ellis et al., 2009).
Total N excretion (g/d) = 6.91 + 0.759 $\times$ ((DMI $\times$ 1 000) $\times$ Dietary N concentration (%)/6.25); (Waldrip et al., 2013).

Although recent models have been developed to predict enteric methane emissions, the model from Ellis et al. (2009) was employed in this study due to its favourable balance between prediction accuracy and the availability of input data. For instance, in a comparative study conducted by Benaouda et al. (2019), the models proposed by Escobar-Bahamondes et al. (2017) and Ramin and Huhtanen (2013) outperformed other predictive models. However, it is important to note that these models require inputs that are not available in our database, such as fat or ether extract concentration in the diet.

Statistical analysis

Statistical analysis and algorithm applications were performed in R (RStudio Core Team, version 1.1.463, 2018). All data were assessed for normality and homoscedasticity using the Lillie test and the Levene test ("Nortest" library), respectively. After confirming the normal distribution of data, differences between nutritional strategies (Multiphase vs commercial diets) were tested via ANOVA (Type III) as shown in the following model:

$$Y_{(ijk)} = \mu + N_i + S_j + D_k + \varepsilon$$

where Y is the dependent variable, μ is the overall mean; N_i is the effect of the nutritional strategy (commercial vs. multiphase diets); S_j is the effect of the study (2019 vs. 2020), D_k is the effect of diet used (concentrate A vs. B), ε_i is the random effect. The results of ANOVA were declared significant when P-value $\leq$0.05. To assess the between-animal variation in animal performances, we computed the coefficient of variation, which is obtained by dividing the SD by the mean value of each performance.

Results

All variables followed a non-normal distribution, except DMI. Also, DMI did not fulfil homoscedasticity within-contemporary group (P < 0.05).

Observed animal performances during the fattening period

As previously mentioned, the two concentrates used herein (A and B) had similar chemical compositions in terms of fibre, starch, energy and protein, and the resulting animal performances differed between them. Table 2 shows that animals fed with Diet A had smaller total DMI ($-$6.8%; P < 0.01), ADG ($-$28%; P < 0.001) and FCE ($-$21.2%; P < 0.001) than animals on Diet B. The DMI difference was mainly due to the greater concentrate intake in Diet B than in A (+6.6%; P = 0.05). Consequently, both energy and protein intake were smaller in Diet A ($-$6.8% on average; P < 0.01) than in Diet B.

Regarding animal performances, animals fed Diet A presented smaller energy ($-$8.7%; P < 0.001) and protein ($-$32%; P < 0.001) requirements than those fed Diet B. In addition, animals fed Diet A showed smaller estimated CH_4 emissions ($-$9.9%; P < 0.001) and estimated N excretions ($-$6.2; P < 0.001). However, there were no differences between the diets in terms of estimated CH_4 yield (MJ CH_4/kg DMI) or N yield (g N/kg DMI). Finally, due to the smaller DMI in Diet A, the daily feeding cost was also smaller ($-$8%; P < 0.01) for Diet A compared to Diet B.

Relationships between animal BW and nutritional requirements

Before proceeding to model the relationships between the main objective variables (Net energy requirements (Mcal/d), metabolisable protein requirements (g/d), dietary concentrations of net energy (Mcal/kg DMI), and metabolisable protein (g/kg DMI)) and BW, we explored the variance explained by random effects to introduce these effects into the model. Table 3 presents the percentage of variance explained by each random experimental factor

Table 2
Observed and estimated average animal performances of crossbred Holstein bulls fed concentrate and straw during the fattening period.

Experimental diets	Concentrate A		Concentrate B	
	Average	CV (%)	Average	CV (%)
Observed animal performances				
Total DMI_{av} (kg/d)	8.06	0.19	8.61	0.15
DMI concentrate (kg/d)	7.39	0.20	7.91	0.16
DMI straw (kg/d)	0.66	0.19	0.71	0.18
BW_0 (kg)	309	0.09	322	0.11
BW_{av} (kg)	394	0.13	445	0.16
Age_0 (d)	223	0.05	220	0.07
Age_{av} (d)	283	0.11	283	0.13
ADG (kg/d)	1.36	0.46	1.87	0.29
FCE (g ADG/g DM)	0.19	0.47	0.24	0.29
NDF intake (kg DM/d)	1.43	0.22	1.63	0.19
Starch intake (kg DM/d)	2.94	0.22	3.04	0.19
Net energy intake (Mcal/d)	14.4	0.19	15.4	0.15
MP intake (g DM/d)	651	0.19	695	0.15
Estimated animal performances				
Net energy for maintenance (Mcal/d)	8.30	0.10	9.08	0.12
Net energy for gain (Mcal/d)	3.01	0.93	4.59	0.49
Total Net energy (Mcal/d)	11.2	0.33	14.0	0.21
MP for maintenance (g/d)	171	0.13	190	0.14
Total MP (g/d)	429	0.19	537	0.17
CH_4 Emissions (MJ/d)	91.2	0.24	103	0.19
CH_4 yield (MJ/kg DMI)	12.2	0.56	12.8	0.39
Total N excretion (g/d)	123	0.19	131	0.15
N excretion yield (g/kg DMI)	16.7	0.21	16.7	0.14
Feeding cost (€/d)	2.64	0.20	2.83	0.16

Abbreviations: DMI = DM intake, ADG = average daily gain, FCE = feed conversion efficiency, MP = metabolisable protein, BW_0 and Age_0 = BW and age at the beginning of the trial, BW_{av} and Age_{av} = BW and age averages during the whole trial.

Table 3
Variance analysis of random effects on nutritional requirements and dietary concentration variables of crossbred Holstein bulls.

Item	Animal requirements		Dietary concentration[1]	
	Net energy (Mcal/d)	Metabolisable protein (g DM/d)	Net energy (Mcal/kg)	Metabolisable protein (g DM/kg)
Variance estimates of random effects				
Study	0.01	0.01	0.01	0.01
Diet within study	0.31	0.31	0.23	0.29
Pen within diet and study	0.08	0.08	0.07	0.06
Animal within pen, diet and study	0.11	0.12	0.29	0.18
Residual	0.49	0.48	0.40	0.46

[1] Dietary energy and protein concentration were calculated by dividing energy and protein requirements by observed feed intake.

for each objective variable. Generally, the diet effect accounted for the largest percentage of variance in all our variables (28%), followed by the effect of the animal (16%) and the pen (7.5%). Finally, the variance explained by the study was not significant for any of our variables. All these effects were introduced into the model following a nested random structure (as detailed in Material and methods section), regardless of the percentage of variance explained.

The relationships between energy and protein requirements and intakes (Mcal/d and g/d, respectively) with BW throughout the fattening period are presented in Table 4. These relationships revealed differences between required and offered energy and protein (Fig. 2), indicating a need to adjust nutrient supply to the requirements. Data of protein intake and requirements were best fitted by a linear model, as the quadratic model did not show significant difference ($P \geq 0.07$) in terms of AIC, BIC, and RMSE. In contrast, both energy intake and requirement data were best fitted by the quadratic model, as indicated by the smaller significant values of AIC and BIC, compared to linear model. The energy requirement increased with BW (over time), although with small linear slope. However, the energy intake model presented a negative coefficient of BW^2, reflecting a slight asymptote at 500 kg of BW. This can be better observed in Fig. 2, which displays both intake and requirement models in the same graph. The comparison between protein intake and requirements highlighted a greater protein intake than protein requirement (+28%, P = 0.001), especially at the end (after 500 kg of BW), where this difference increased up to 32%. For energy, the difference between intake and requirements was smaller than that observed for protein. For instance, energy intake was greater than required (+16%, P = 0.001). However, from 350 to 450 kg of BW, this excess intake could reach 26%.

Table 5 presents the relationships between the dietary energy or protein concentration required (Mcal/kg DMI or g MP/kg DMI, respectively) and animal BW. The energy and protein concentrations required were calculated by dividing the daily requirements (Mcal/d or g MP/d, respectively) by the observed intake. As in the previous table, different fits to the data were compared (linear, quadratic, and exponential). The required MP concentration data were best-fitted by an exponential decay model that decreases with BW increase. This decrease in MP concentration reached an asymptote in the curve at 487 kg of BW (corresponding to 59 g MP/kg DMI). In the case of energy concentration, the data were best fitted by the quadratic model, with the energy concentration required being slightly greater at the beginning (280–320 kg BW). These models are represented in Fig. 3, where both the dietary energy and protein concentration required across the fattening period were plotted. The results indicated that the daily energy and protein requirements (Mcal/d and g/d, respectively) increased with

Table 4
Relationships between daily energy and protein intake and requirements of crossbred Holstein bulls with the BW of animals across their fattening period.

Eq. no.	Type of model	Y variable	X variable	Equation[1]	AIC	BIC	RMSE	*P*-value[2]
1	Linear model	Protein requirements (g/d)	BW (kg)	278 + 0.4954 × BW	2 339	2 346	80.7	*P* = 0.32
2	Quadratic model			533–0.744 × BW + 0.001492 × BW^2	2 335	2 342	81.3	
3	Linear model	Protein intake (g/d)	BW (kg)	448 + 0.5689 × BW	2 281	2 288	53.3	*P* = 0.07
4	Quadratic model			159 + 2.024 × BW − 0.00178 × BW^2	2 268	2 275	54.4	
5	Linear model	Energy requirements (Mcal/d)	BW (kg)	8.739 + 0.009518 × BW	8 914	8 981	2.06	*P* < 0.01
6	Quadratic model			8.97 + 0.0076 × BW + 0.000003 × BW^2	8 789	8 862	2.05	
7	Linear model	Energy intake (Mcal/d)	BW (kg)	9.90 + 0.01261 × BW	7 704	7 771	1.19	*P* < 0.01
8	Quadratic model			3.52 + 0.04476 × BW −0.0000394 × BW^2	7 567	7 639	1.18	

Abbreviations: Eq = equation, AIC = Akaike Information Criterion, BIC = Bayesian information criterion.
[1] Equations used animal BW (kg) as dependent variable.
[2] Anova analysis was conducted to compare the AIC and BIC values between the linear and quadratic models. The quadratic model is considered the best-fitted model only if the AIC and BIC are significantly (*P* < 0.05) smaller than those of the linear model.

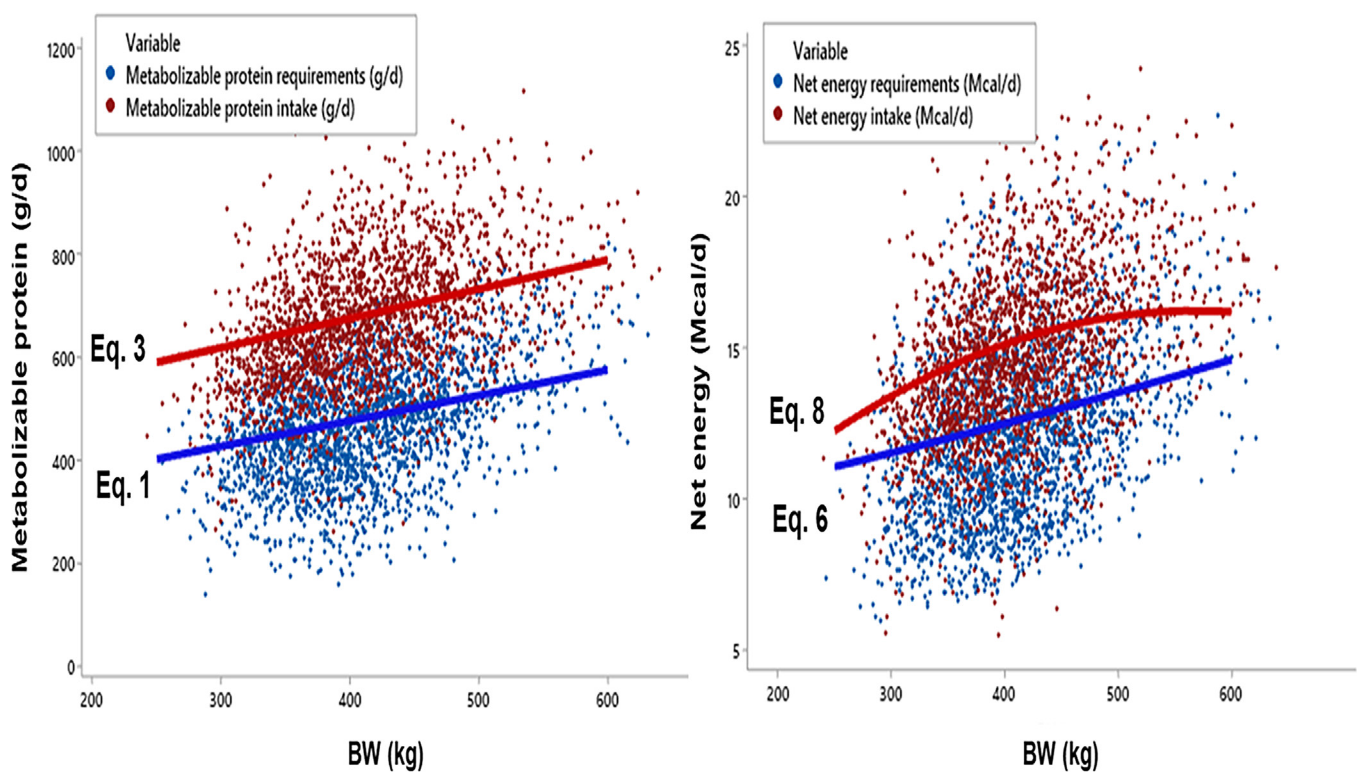

Fig. 2. Relationships between intake and requirements in terms of energy and protein, and BW during the fattening period of crossbred Holstein bulls. Each point represents each individual animal for intake (blue) and requirements (red), considering the random effects of the study, the diet, the pen and the animal. The blue and red lines show the best model's equations for each parameter (see the equation coefficient in Table 4). Abbreviation: Eq. = Equation.

Table 5
Statistical relationships between intake and requirements in terms of energy and protein parameters of crossbred Holstein bulls across the BW of animals during their fattening period.

Eq. no.	Type of model	Y variable	X variable	Equation[1]	AIC	BIC	RMSE	*P*-value[2]
9	Linear model	Dietary protein concentration (g/kg DMI)[3]	BW (kg)	62.2 + 0.0004112 × BW	1 498	1 505	9.99	*P* < 0.01
10	Quadratic model			95.14 − 0.157 × BW + 0.000185 × BW^2	1 497	1 504	9.95	
11	Exponential decay model			32 120 × exp[−0.026 × BW] + 59.9	485	487	2.33	
12	Linear model	Dietary energy concentration (Mcal/kg DMI)[3]	BW (kg)	1.79 − 0.0004 × BW	933	1 000	0.24	*P* = 0.02
13	Quadratic model			2.26 − 0.0026 × BW + 0.000003 × BW^2	818	890	0.25	

Abbreviations: Eq = equation, AIC = Akaike Information Criterion, BIC = Bayesian information criterion.
[1] Equations used animal BW (kg) as dependent variable.
[2] Anova analysis was conducted to compare the AIC and BIC values between the linear and quadratic models. The quadratic model is considered the best-fitted model only if the AIC and BIC are significantly (*P* < 0.05) smaller than those of the linear model.
[3] Dietary energy and protein concentration were calculated by dividing energy and protein requirements by observed feed intake.

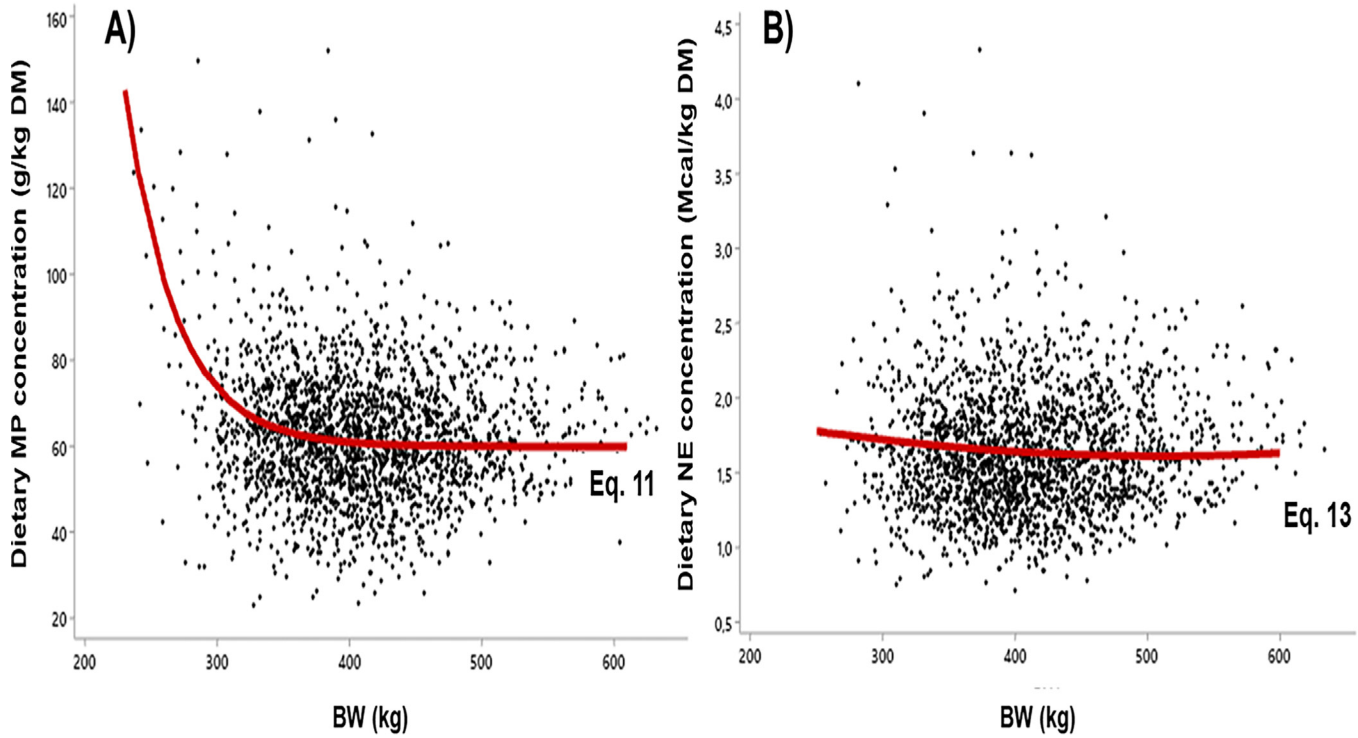

Fig. 3. Modelling the dietary concentrations of metabolisable protein (A) and net energy (B) required in crossbred Holstein bulls during their fattening period. The dietary concentration required was estimated as daily requirements of metabolisable protein and net energy divided by the observed DMI. Black points represent individual values of animals, while continuous red lines show the best regression model for each parameter (see the equation coefficients in Table 5). Regression models considered the random effects of the study, the diet, the pen and the animal. Abbreviations: NE = net energy, MP = metabolisable protein, DMI = DM intake, Eq. = Equation.

BW because of an increase in the animal's overall needs. However, given that the DMI also increases with BW, the dietary energy and protein concentration should decrease in a quadratic and exponential pattern.

Optimal change points of dietary energy and protein concentration across the fattening period

After selecting the best-fit models of dietary protein and energy concentration required (Equations 11 and 13 in Table 5, respectively), the optimal change points were determined, by minimising the area between two curves. The change points are expressed as BW during the fattening period when it would be suitable to adjust the NE and/or MP concentration of the diet. The optimal change point could be determined using the NE or MP concentration model. However, given that the NE concentration model was slightly quadratic (almost linear and constant), the optimal change points were identified using only the MP concentration model. Once the BW at which to change MP concentration was identified, the corresponding NE concentration required was also used to reformulate the new diets.

Fig. 4 shows the dietary protein concentration required (blue line) and the protein concentration of the commercial diet used in our studies (black line). The area between these two lines (in white) reflected the excess metabolisable protein supply of 8.090 g during the fattening period. The red area represents the excess supply after changing the diet (MP concentration adjustment). For simplicity and applicability in commercial beef cattle farms, we limited diet changes to two. The optimal points for these two changes were at 312 kg and 387 kg of BW. Therefore, the fattening period could be divided into three phases; <312 kg, 312–385 kg, and >385 kg of BW. Applying dietary changes at these two BW points, the excess MP could be reduced to 1.080 g instead of 8.090 g (red area in Fig. 4). For our animals (crossbred Holstein bulls), the three-phase diet should have the following concentrations: 88 g MP/kg DM and 1.75 Mcal NE/kg DM (under 312 kg of BW); 70 g MP/kg DM and 1.70 Mcal NE/kg DM (BW between 312 and 385 kg); 61 g MP/kg DM and 1.65 Mcal/kg DM (over 385 kg of BW).

Comparing commercial vs. multiphase diet in beef cattle production

Once dietary energy and protein concentration at these three new phases across the fattening period of our dairy-beef crossbreed Holstein bulls were estimated, new diets were formulated based on the newly suggested protein and energy concentrations (Table 6). These new formulations were adapted to meet animal requirements while minimising feeding cost. The main difference between the new and commercial diets used in the *in vivo* trials resulted in a decreased estimated N excretion (>75 g MP/kg DM), which conducted to the inclusion of other high-fibre ingredients, such as granulated wheat straw instead of protein sources. Consequently, the new diets, on average, presented both greater starch (+19%) and fibre (+26%) values than commercial diets. Next, the comparison of animal performance, environmental impact, and economic profitability between commercial and multiphase diets are shown in Table 7. We first compared the multiphase vs conventional diet at each phase before comparing both diets during the entire fattening period.

As the first-phase fattening (<312 kg of BW) was fairly similar between the commercial and multiphase diet, we decided to only compare the second and third phases.

In the second phase (312–385 kg of BW), animal performance, intake, weight gain, and efficiency) remained similar between commercial and multiphase diets ($P > 0.16$). However, the feeding cost was smaller (−7.6%, $P < 0.001$) in the multiphase diet com-

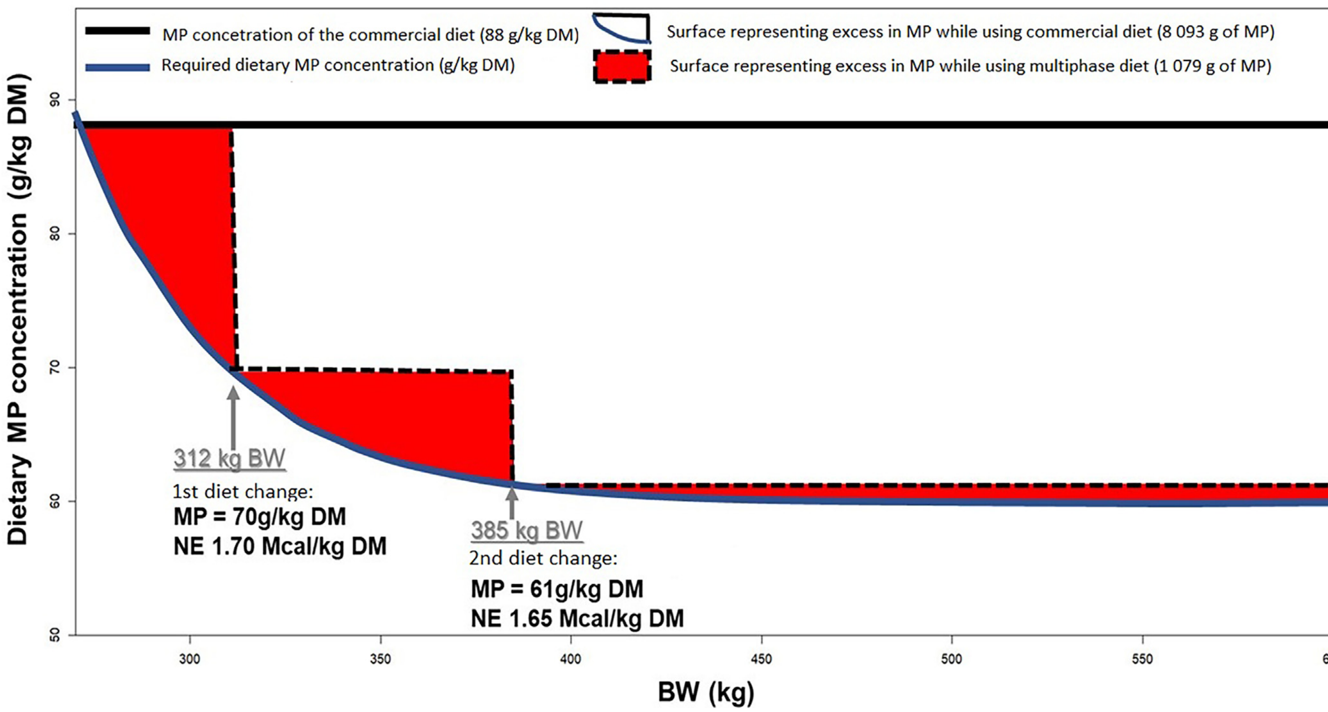

Fig. 4. Main change points identified in the dietary protein concentration required by crossbred Holstein bulls (blue line) using the optimisation of the area between two curves. Upper black line represents dietary protein concentration of the commercial unique diet offered. White area shows the difference between dietary protein concentration required and offered representing the theoretical excess of protein given. Main changing points identified three different fattening phases; <312 kg, 312–385 kg, and > 385 kg of BW. Red surface shows the theoretical difference between offered and required once the two changes were applied. Abbreviations: NE = net energy, MP = metabolisable protein.

Table 6
Composition of the reformulated diets according to the new estimated dietary energy and protein concentration required for crossbred Holstein bulls.

	New reformulated diets	
BW intervals (kg)	312–385 (phase 2)	385–550 (phase 3)
Ingredient composition, % inclusion on DM basis		
Corn	56	56
Granulated wheat straw	17	23
Wheat middlings	22	19
Sunflower meal	3.5	0.0
Calcium carbonate	1.3	1.3
Salt	0.4	0.4
Minerals and vitamin mix[1]	0.2	0.2
Dietary chemical composition (g/kg of DM)		
Organic matter	890	890
CP	101	87.3
NDF	296	320
ADF	156	179
Starch	480	469
Starch/NDF (g/g)	1.62	1.46
Net Energy (Mcal/kg DM)	1.76	1.70
MP (g/kg of DM)[2]	70.1	61.1
MP/Net energy (g/Mcal)[2]	39.7	47,5

[1] Minerals and vitamin mix: 5% P, 25% Ca, 8% Mg, 0.2% Na, vitamin A (30 000 000 IU/kg), vitamin D3 (1 000 000 IU/kg) and vitamin E (30 000 mg/kg).
[2] Metabolisable Protein

pared with the commercial diet. The estimated enteric CH_4 emissions were greater (+38.3%, $P < 0.001$) in animals fed the multiphase diet compared, whereas the estimated N excretions were smaller (−14%, $P < 0.001$).

Regarding the third phase (>385 kg of BW), the gain was similar between the two diets but intake was greater (+9.2%, $P < 0.001$) in the multiphase diet than in the commercial one, resulting in smaller feed efficiency (−13.5%, $P < 0.001$). The environmental impact in this third phase was similar to that observed in the second phase, presenting even greater estimated CH_4 emissions (+46%, $P < 0.001$) but smaller estimated N excretion (−19%, $P < 0.001$) in the multiphase diet compared to the commercial diet, which again could be likely related to the increase in dietary fibre content of the concentrate. Finally, feeding costs were also smaller (−3%, $P > 0.001$) in multiphase than in commercial diets as a result of the changes in concentrate formulas.

Considering the entire fattening period (the three phases herein evaluated), it was estimated that the duration remained similar between the multiphase and commercial diets (202 ± 13 d, $P > 0.26$), resulting in an economic saving of 4% (29 €/animal) with the multiphase diet. Regarding the environmental impact of the multiphase diet in this entire fattening period, a reduction in terms of N excretion (−16%, $P < 0.001$, 210 g N/animal) but with an increase in CH_4 emissions (+44%, $P < 0.001$, 331 g CH_4/animal) was estimated.

Discussion

The present study is a retrospective simulation in which we analysed animal performance of young fattening crossbred Holstein bulls in order to investigate whether adopting a multiphase diet strategy could improve both the economic and environmental impact of cattle production, similar to what has been demonstrated in pig production (Pomar et al., 2014; Andretta et al., 2016). This retrospective study included data from two *in vivo* trials involving crossbred Holstein bulls fed balanced diets (straw and concentrates A or B). Individual trajectories of NE and MP requirements were modelled, and the overall relationship between these requirements and the BW was established. By undertaking this comprehensive modeling, we were able to estimate the difference between the requirements (NE and MP) and the observed intake,

Table 7
Comparison of observed and estimated animal performances of crossbred Holstein bulls between the commercial and the multiphase diets by intervals of BW (phases).

Observed/estimated animal performances	Commercial diet		Multiphase diet		*P*-value
	Mean	CV (%)	Mean	CV (%)	
Second phase; 312–385 kg					
Total DMI_{av} (kg/d)	7.67	0.17	7.73	0.54	0.20
ADG (kg/d)	1.50	0.37	1.65	0.58	0.16
FCE (g ADG/g DMI)	0.217	0.36	0.214	0.39	0.44
CH_4 Emissions (MJ/d)	86.5[b]	0.21	140[a]	0.09	<0.001
CH_4 by intake (MJ/kg DMI)	11.3[b]	0.23	18.2[a]	0.13	<0.001
Total N excretion (g/d)	118[a]	0.16	102[b]	3.70	<0.001
N excretion by intake (g/kg DMI)	15.4[a]	0.95	13.2[b]	0.08	<0.001
Feeding cost (€/d)	2.51[a]	0.42	2.31[b]	0.08	<0.001
Third phase; 385–600 kg					
Total DMI_{av} (kg/d)	8.66[b]	0.17	9.53[a]	0.91	<0.001
ADG (kg/d)	1.57	0.35	1.62	0.21	0.13
FCE (g ADG/g DMI)	0.201[a]	0.35	0.174[b]	0.29	0.02
CH_4 Emissions (MJ/d)	101[b]	0.21	191[a]	0.21	<0.001
CH_4 by intake (MJ/kg DMI)	11.7[b]	1.19	20.1[a]	0.17	<0.001
Total N excretion (g/d)	132[a]	0.16	108[b]	9.73	<0.001
N excretion by intake (g/kg DMI)	15.2[a]	0.95	11.33[b]	0.06	<0.001
Feeding cost (€/d)	2.84[a]	0.49	2.76[b]	0.26	<0.001

Abbreviations: ADG: Average Daily Gain, FCE: Feed Conversion Efficiency.
[a,b] Values with different letters within the same row are significantly different ($P < 0.05$).

identify optimal moments for dietary adjustments, and simulate the economic (diet cost) and environmental benefit (N excretions and enteric CH4 emissions) of implementing these adjusted diets.

The results demonstrated that adjusting dietary formulation to meet nutritional requirements at 312 kg and at 385 kg of BW decreased feeding costs and estimated N excretion; however, it resulted in an increase in estimated CH4 emissions.

Factors increasing variation in animal requirements calculation

The calculation of animal requirements relies mainly on animal performance, and therefore, variations in performance have a direct impact on our subsequent requirement calculation. Among the random factors analysed in our mixed model of NE and MP requirements, the diet was the main source of variations. For instance, animals fed diet B presented slightly greater intake of concentrate than animals fed diet A, with no differences in terms of straw intake. This could be due to three possible reasons:

(1) Difference in diets formula: even if the chemical composition was similar, Diet B had larger inclusion of ingredients considered as "highly palatable" for cattle, such as soybean meal or corn (Baumont, 1996; Miller-Cushon et al., 2014).
(2) Difference in animal genetics, which can differ between periods and studies because it depends on the moment of the animal's purchase. In the case of crossbred Holstein bulls, genetics can vary due to the diverse origins and dam crosses (Edwards et al., 2011). These genetic differences may lead to different performances, such as ADG, even between animals with similar initial BW or age ($P > 0.07$). It is worth noting that the model used to estimate body composition is dated and may have limitations since animal genetics have evolved over the last 20 years due to genetic selection.
(3) Differences in health status, which is also related to animal's origin (transport and sanitary status of the farm). Dairy beef calves are susceptible to illnesses like bovine respiratory disease during their transition from the rearing phase. These illnesses can affect their performance to different degrees based on lesion severity and increasing BW variability among animals belonging to the same fattening batch (Taylor et al., 2010). Consequently, the greater environmental impact of animals fed Diet B was mostly due to their greater intake and excretion than animals fed Diet A. It is well known that DMI is the main driver of enteric CH_4 emissions (Charmley et al., 2015, Benaouda et al., 2019), and the economic profitability of cattle production (Richardson et al., 2020).

All these differences increased the between-animal variation of performance, and consequently, the variation in the calculation of an individual's requirements. For instance, the diet effect was the most important factor to explain variation (≈30%) of both daily requirements and the required dietary concentrations. The composition of ingredients in the diet plays a crucial role in determining both the total energy and protein absorbed in the intestine and the quantity of dietary energy and protein allocated for maintenance or growth (Geay, 1984). In addition, other dietary components, like fibre, are directly used in the calculation of animal requirement, such as the estimation of maintenance protein (INRA, 2018). The variance explained by the pen effect was small. This is partly due to the fact that animals were sorted by BW, a practice that tends to minimise between them (Cruz et al., 2010). Finally, it is worth mentioning that the variance explained by the study was not significant for any of our variables. This could be attributed to two possible reasons. Firstly, the random effect of study might be confounded with the other random factors in the analysis. Secondly, even though the studies differed by year, experimental facilities, workers, and protocols remained consistent across all the studies.

Modelling an individual animal's requirements and dietary concentration required

As expected from a biological perspective, the daily energy and protein requirements of animals increased over time due to their rising BW and, consequently, their increasing maintenance demand (Johnson et al., 2012). In addition, variation in animal's requirements across time could be also attributed to the change in body composition, particularly protein and fat deposition even within the same breed (Diaz et al., 2001). Following the common Gompertz's curve, specifically the last mean of this curve (Winsor, 1932), protein deposition and, by extension, protein requirements, increased proportionally more than energy requirements during the early growing phase (Fox et al., 1992). This observation may explain why protein requirements better fitted by a simple linear model, while energy requirements are better represented by a quadratic model. This phenomenon was also observed by Bruce (1986), who proposed a quadratic equation for both maintenance and gain energy requirements.

These differences, added to the DMI curve across the fattening period, may explain why the required dietary protein concentration fitted better to an exponential decay model, indicating significantly large concentrations of MP when animals were in the first stage of growth (<300 kg BW, Fig. 3). In contrast, the increase in energy requirement might be more aligned with the rise in feed intake, resulting in flat linear model of energy dietary concentration. It is worth mentioning that concentrate intake was recorded on a daily and individual basis, whereas straw intake was estimated as collective straw intake per pen divided by the number of animals. This method of estimating straw intake may have a slight influence on the modelled intake estimates.

Multiphase diet as an effective nutritional strategy to improve beef cattle production efficiency

After conducting the modelling of animal's requirements and dietary energy and protein required, we identified the optimal changing points, allowing us to establish three distinct phases. Given that the initial phase (BW < 312 kg) was consistent between the commercial and multiphase diets, we opted to directly compare the second phase (312–386 kg BW) and the third phase (385–550 kg BW) against the commercial diet.

In the second phase, we observed notable differences, particularly a reduction in feeding costs and estimated N excretion in the multiphase diet compared to the commercial diet. However, there was an increase in estimated methane emissions. These differences can largely be attributed to variations in dietary formulation. The multiphase diet had smaller protein and energy concentrations in comparison to the commercial diet, which is associated with reduced N excretion. However, the decrease in required energy concentration led to reformulate diet, involving the substitution of part of the cereals (barley and corn) with fibre-rich ingredients with lower energy content, such as wheat middlings or granulated straw. Consequently, the multiphase diet exhibited lower costs but also a higher dietary fibre content than the commercial diet, which could explain the potential increase in methane production and emissions (Hindrichsen et al., 2005).

Comparisons between multiphase and commercial diets in the third phase yielded results similar to the second phase. However, as the third-phase diet had smaller concentration of NE and MP, feed efficiency was lower and feed intake was larger, as compared to the commercial diet.

When considering the overall comparison of multiphase vs commercial diets throughout the entire fattening period, the main findings indicated significant economic savings and N excretion reduction, with an increase in enteric methane emissions. Other precision nutrition techniques (nutritional grouping) were evaluated in dairy cows and presented an economic saving of 35$ per animal and per period (Barrientos-Blanco et al., 2020) and a reduction in N excretion (Kalantari et al., 2016). Considering that precision nutrition techniques aim to better match nutrient intake and requirements, these economic saving and reduced N are expected outcomes. However, it is important to note that the increase in estimated methane emissions resulting from dietary formulation modifications, particularly greater fibre inclusion, represents a significant challenge for this nutritional strategy. This concern was already pointed out by Dijkstra et al. (2011), who explored nutritional strategies to reduce N excretion in cattle and also observed an increase in methane emissions. They emphasised the need to comprehend the trade-off between N excretion and enteric methane production at the individual animal level to provide accurate data for farm-level decisions.

Furthermore, we suggest that other nutritional strategies, such as incorporating oils or seeds rich in polyunsaturated fatty acids or using feed additives to reduce methane emissions, should be explored to maintain low energy concentration in the concentrate while decreasing both nitrogen and methane emissions (Beauchemin and McGinn, 2006; Norris et al., 2020; Bačėninaitė et al., 2022). In future *in silico* studies, multi-objective optimisation algorithms can be implemented to find the best trade-off between targeted objectives, such as minimising enteric CH_4, N excretion, feed cost or optimising expected meat value . . .etc.

In addition, further *in vivo* studies are necessary to validate the conclusions drawn from our simulation. *In vivo* studies can provide more accurate animal performances than predictive models, especially ADG trait, which is closely linked to protein intake (Tedeschi et al., 2015). Furthermore, in the present study, we did not measure or predict the carcass characteristics in commercial vs. multiphase diet. Changes in diet composition can lead to variations in carcass quality, which in turn may impact economical profitability.

Conclusion

This study highlights the potential of multiphase diets as a valuable tool for improving the efficiency of beef cattle production, yielding economic benefits and reduced nitrogen excretion. However, there is a need for a holistic approach that considers other environmental impacts, particularly methane emissions. Further *in vivo* studies are crucial to validate these findings and provide more comprehensive insights into the practical implications of implementing multiphase diets in beef cattle production.

Ethics approval

All calves used were managed following the principles and guidelines of the Animal Care Committee of the Institut de Recerca i Tecnologia Agroalimentàries (RD 53/2013; project number: 10478).

Data and model availability statement

None of the data was deposited in an official repository. The data/models that support this study's findings are available from the authors upon request and after authorisation by all authors.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

Acknowledgements

The author M. Benaouda would thank the "region Bourgogne-Franche-Comté" for the financial support. The authors M. Devant, S. Marti, and L. Llonch belong to the Consolidated Research Group of Sustainable Animal Husbandry, funded by the AGAUR (Generalitat de Catalunya; ref. 2021 SGR 01552). IRTA also thanks the support of the Generalitat de Catalunya through the CERCA Program.

Financial support statement

This research received a financial support from the "region Bourgogne-Franche-Comté" in the framework of the project NTechBV, grant no. 2021-y-09053.

Transparency declaration

This article is part of a supplement entitled 10th International Workshop on Modelling Nutrient Digestion and Utilization in Farm Animals (MODNUT) supported by the Department of Agricultural Sciences, University of Sassari, Sardinia, Italy.

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