Evaluation and Refinement of the French Protein System (PDI) under Irish Conditions

End of Project Report 4940

Project Team:

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Summary

The CP and DM degradability of grazed grass (between April and October, inclusive) and grass silage samples (differing in cut number and treatment) was determined using the *in situ* technique and the results obtained were used to calculate the PDIE and PDIN values. The degradability data on 12 concentrate ingredients from a previous study (Woods, 2000) were used to estimate PDIE and PDIN values for these feedstuffs. The mean PDIE and PDIN values determined for grass were 81 g/kg and 127 g/kg DM, respectively and for grass silage were 60 g/kg and 94 g/kg DM. Regression analysis resulted in equations to predict the PDIN of samples of grazed grass (PDIN = 3.8 + 0.628 CP) (R^2= 0.999) and grass silage (PDIN = 5.9 + 0.605 CP) (R^2= 0.997), and the PDIE of grazed grass (PDIE = 181.4 - 0.104 NDF - 0.195 ash - 0.047 OMD) (R^2= 0.987) and grass silage (PDIE = 27.7 + 0.083 DMD - 0.147 CP) (R^2= 0.812). The PDIE and PDIN values of 11 of the 12 concentrate ingredients were similar to those used in the French Tables but the maize distillers' grains in this study and those used in France would appear to be quite different products.

The response to PDIE and PDIN in the diet of lactating cows was evaluated and the effect of better balancing the PDIE and PDIN supply on the efficiency of N utilisation was assessed. Twenty autumn calving cows were blocked in a complete Latin Square design and assigned to four different diets varying in PDIN and PDIE content. Each of the four treatments consisted of a concentrate, maize silage and grass silage in the proportions 37:38:25 on a DM basis. There were 4 periods of 4-week duration each. Diet A contained 92 g/kg DM of PDIE and 116 g/kg DM of PDIN. Diet B contained 103 g/kg DM of PDIE and 122 g/kg DM of PDIN. Diet C and D over supplied PDIN at 137 g/kg and 153 g/kg, respectively relative to PDIE at 111 g/kg DM. Dry matter intake increased significantly with the excess dietary PDIN relative to PDIE but there were no significant differences in milk yield and composition. Decreasing the supply of PDIE in the diet (i.e. diet A vs. B) resulted in no significant effect on milk or constituent yields but did significantly reduce the efficiency (kg milk / kg DMI) of milk production. There was also a significant reduction in the efficiency of milk produced per kg DMI with increasing dietary concentrations of PDIN and increasing PDIN: PDIE balance (B>C>D). Increasing the dietary PDIN from 122 to 153 g/kg DMI increased urine N (+54%), faecal N (+11%) and plasma urea concentrations (+75%). The results indicate that the optimum concentration of dietary PDI is approximately 103 g/kg DM for cows producing about 35 kg of milk per day. A better balance between PDIE and PDIN supply improves the efficiency of conversion of DM to milk and dietary protein.

Introduction

It is now well recognised that crude protein or digestible crude protein are neither adequate or biologically sensible measures of the value of dietary protein for ruminants. Consequently most European countries have developed alternative systems based on the concept of metabolisable protein. The French protein (PDI) system is based on this concept and estimates the quantity of amino acids absorbed in the small intestine from the dietary protein undegraded in the rumen and microbial protein synthesised in the rumen. Teagasc, University College Dublin, the Department of Agriculture and Food and the Feed Industry have decided to adopt the French system of feed evaluation incorporating the energy, protein (PDI) and intake sub-systems.

Typically grass and grass silage based diets supply an excess of N over energy to the rumen resulting in the inefficient conversion of feed N to milk protein. A central principle of the PDI system is the balancing of energy and protein in the rumen and thus formulating diets using this system should result in improved efficiency of utilisation of feed N and potentially a reduction in the level of supplementary protein required. In order to have confidence in implementing this system in Ireland it is essential to have data on the PDIE (true protein absorbable in the small intestine when energy is limiting in the rumen) and PDIN (true protein absorbable in the small intestine when N is limiting in the rumen) values for Irish grass and silage. Also it is important to have data on the effect on milk production and composition and on efficiency of N use of formulating diets with the system where PDIE and PDIN are better balanced.

Experiment 1. The PDIE and PDIN content of Irish grass silage and grazed grass and the most important concentrate ingredients in Ireland

Introduction

The PDI system provides values for feedstuffs which reflects the true protein absorbed in the small intestine and it aims to balance the nitrogen and energy available in the rumen for microbial protein synthesis. Feedstuffs are assigned two values, PDIN and PDIE. This system, like other modern protein rationing systems, requires accurate measurement of the characteristics of feed protein degradation in the rumen. In all the protein rationing systems presently in use, *in situ* degradation is the most commonly used method to characterise the ruminal degradability of dietary N.

The objectives of this experiment were to measure the PDIE and PDIN contents of Irish grass silage samples and of grass samples taken throughout the grazing season, and also to determine the PDIE and PDIN of 12 concentrate ingredients commonly used in Ireland, whose degradability had previously been measured (Woods, 2000).

Materials and methods

Steers and diet

Three, two-year-old Holstein Friesian steers with an average body weight of 585 kg (s.d. 18kg) were fitted with permanent rumen cannulae (120 mm internal diameter, Ankom Technology Corp, New York). They were offered a diet (75:25 on a DM basis) of second-cut grass silage and concentrates (barley 410 g/kg, unmolassed beet pulp 410 g/kg, soyabean meal 100 g/kg, molasses 55 g/kg, minerals/vitamins 25 g/kg) in a ratio of 75:25 (DM basis) and at a rate of 15 g DM /kg body weight. Silage was fed once daily at 0930h, while concentrates were fed in two equal parts at 0930h and 1730h.

Samples

Ten grass silage samples differing in cutting date (May-July), cut number (1st and 2nd cut), treatment (addition of additive or not) and origin (samples taken from six different research farm) were used. Silage samples were obtained by core sampling the entire depth of the pit and were oven-dried at 40°C for 48hrs.

Seven grazed grass samples, from the Curtin's Research Farm, Moorepark were used, each one representing the months April to October inclusive. Four strips (0.95 m x 5 m) of herbage were cut above 4 cm from a number of pre-grazed paddocks during each month. The swards contained 100% perennial ryegrass *(Lolium perenne)*. The cuts were bulked according to paddock and a sub-sample of each bulk was freeze-dried and composited by month.

The dried grass silage and grass samples were milled through a 1mm sieve.

Degradability Procedure

Heat-sealed nylon bags (Ankom Technology Corp, New York, Catalogue no. R510) with internal dimensions of 9.5 x 5.0 cm and a porosity of 50 μm ± 10, were filled with 2.0 g (1.996 - 2.004g) of sample for incubation (weight to surface ratio was ~21 mg/cm² on a DM basis).

Twelve bags (6 removal times x 2 replicates) for each feedstuff were placed into larger (13 x 24 cm) mesh bags with a mesh size of ~300 μm and weighed down in the rumen with a bottle filled with sand (~575g). Bags were placed in the rumen of each steer before the morning feed at 0930h. Two bags containing each feedstuff were removed from each steer at 2, 4, 8, 12, 24, and 48h after incubation.

Each of these bags was subjected to mechanical pummelling (Lab Blender 400 Stomacher) for 5 min in 5 ml of an *in vitro* buffer (4g of ammonium bicarbonate (NH_3HCO_3) and 35g of sodium bicarbonate ($NaHCO_3$) per litre of distilled water).

After mechanical pummelling bags were then frozen at –18ºC until analysis. Before analysis, bags were thawed in cold water and placed in a twin-tub washing machine (Hotpoint 1464) for 30 min. Water was changed after each of three 10-min wash cycles. Samples were dried at 60ºC for 48 hrs and were weighed to calculate DM disappearance. Crude protein concentration in the samples was measured using a LECO FP 428 N analyser.

Effective degradability (ED) of the feed DM and CP in the rumen was calculated using an INRA model (Michalet-Doreau *et al.,* 1987) with an assumed rate of passage of 6 %. The PDI values were calculated using the equations of Vérité and Peyraud. (1989). Data were analysed by forward regression using the PROC REG procedure of SAS to find the relationship between the PDIN and PDIE values and the chemical composition of the grass silages and grazed grasses.

Results

Degradability and PDI values of grass silages

The mean chemical composition of the silages classified as first cut without additive, first cut with additive and second cut is shown in Table 1.

Table 2 shows the degradability constants (a, b and c) and the ED values for the grass silages. Even though there was a relatively high mean ED of CP (~81%) the ED of DM (~55%) was low as there was a high b (slowly but potentially degradable) fraction (~65%) present. The ED of DM for the additive treated silage is lower than the untreated silage, which is related to the lower digestibility of the samples used.

Table 1. The mean chemical composition of first cut, first cut plus additive and second cut grass silage samples incubated in the rumen for the estimation of degradability (g/kg DM unless otherwise stated).

	1 st cut no additive	1st cut + additive *	2 nd cut
n	2	5	3
Mean Cutting Date	26 May	28 May	20 July
Dry Matter (g/kg)	166	182	251
Crude Protein	171	144	133
Ash	97	80	73
Neutral Detergent Fibre	511	594	578
Acid Detergent Fibre	345	394	393
Dry Matter Digestibility (g/kg)	754	613	658
Organic Matter Digestibility (g/kg)	743	597	651
Digestible Organic Matter in the Dry matter	670	549	602

* 85 % Formic Acid (Add Safer, Interchem, Dublin 10) n = 4

Molasses (Premier Molasses, Foynes, Co. Limerick) n = 1

Table 2. Mean degradability constants (a, b and c) and calculated effective degradabilities (ED) (%) of first cut, first cut plus additive and second cut grass silage.						
	Mean cut date		a	b	c	ED
1st Cut	26 May	DM	38.3	60.4	0.03	59.7
n=2		CP	76.1	20.7	0.04	84.7
1st Cut Additive	28 May	DM	30.6	66.6	0.03	52.9
n=5		CP	69.2	25.6	0.05	81.1
2nd Cut	19 July	DM	29.7	66.8	0.04	53.1
n=3		CP	65.2	29.0	0.06	78.5

The PDIN values of the grass silages ranged from 86 to 109 g/kg, while the PDIE values ranged from 56 to 67 g/kg (Table 3). When comparing the PDI values with those in the French tables (Andrieu *et al.,* 1989–Feed Nos. 385-404), Irish first cut silage, with a higher CP content (171 vs. 137 g/kg DM), had a similar PDIE content (67 vs 66 g/kg DM) at a similar OMD content (743 vs. 746 g/kg), but a much higher PDIN value (109 vs. 80g/kg DM). Second cut grass silage had a similar PDIE content (64 vs 65 g/kg DM) as given in the French tables, at a similar CP concentration, but a slightly lower OMD content in this study (651 vs. 696 g/kg). The PDIN value was lower in the French tables (78 vs. 86 g/kg DM). Second cut grass silage had a slightly lower PDIE value than the first cut (64 vs. 67 g/kg DM) and a higher value than first cut plus additive (64 vs. 56 g/kg DM). There was little difference between the PDIE and PDIN of first and second cut grass silage in the French tables. There is a large difference between PDIN values for first and second cut silages in this study (109 vs. 86 g/kg) which is related to the CP of the samples. The results show that the additive treated silages (molasses and formic acid) tended to have lower PDIE and PDIN values in this trial associated with the lower digestibility of the silage compared to first cut without additive (670 vs. 602 g/kg DM). No inference can be made about the use of additives or the differences between first and second cut silage from these data because of the low sample numbers and the fact that the silages were made from different swards, etc. I think equations should go in this section and be highlighted.

The relationship between the PDI values and the chemical composition of grass silage was determined by regression analysis. Including ash, NDF, DMD and CP in the regression model, (these analyses are available in most grass silage analysis reports), gave the following prediction equations for PDIE (R^2= 0.812) (equation 1) and PDIN (R^2= 0.998) (equation 2) in grass silage:

Equation 1. PDIE = 27.7 + 0.083 (DMD) - 0.147 (CP)

Equation 2. PDIN = 6.84+ 0.602 (CP) + 0.032 (ash) - 0.005 (DMD)

Crude Protein is a major variable in the prediction of PDIN. Regressing PDIN on CP only, resulted in the following simple equation for grass silage (R^2 = 0.997) (equation 3):

Equation 3. PDIN = 5.94 + 0.605 (CP)

Table 3. The mean [1]PDI values (g/kg DM) of first cut, first cut plus additive and second cut grass silage.

	1 st cut	1st cut + Additive	2nd cut
PDIA	17	19	20
PDIME	50	37	44
PDIMN	92	73	66
PDIE	67	56	64
PDIN	109	92	86

[1]PDIA = The dietary protein undegraded in the rumen, but truly digestible in the small intestine

PDIME = the amount of absorbable microbial protein that could be synthesised in the rumen, when rumen fermentable energy (organic matter) is limiting

PDIMN = the amount of absorbable microbial protein that could be synthesised in the rumen, when degradable nitrogen is limiting

PDIE = true protein absorbable in the small intestine when rumen fermentable energy (organic matter) is limiting microbial protein synthesis in the rumen

PDIN = true protein absorbable in the small intestine when degradable N is limiting microbial protein synthesis in the rumen

Degradability and PDI values of grazed grass

The chemical composition of the 7 grass samples used for the estimation of DM and CP degradability is shown in Table 4. The CP content of the grass decreased as the season progressed from April to July but increased again from August to October. The fibre content of the samples increased as the season progressed, and is associated with a reduction in the digestibility.

Table 4. Chemical composition of grazed grass (g/kg DM unless stated otherwise)

	April	May	June	July	August	September	October
Dry Matter (g/kg)	182	184	182		191	165	172
Crude Protein	222	166	176	169	189	203	251
Ash	95	81	85	82	83	86	108
Neutral Detergent Fibre	400	403	423	425	464	427	506
Acid Detergent Fibre	231	241	256	254	266	253	297
Dry Matter Digestibility (g/kg)	837	855	831	812	778	814	742
Organic Matter Digestibility (g/kg)	830	832	816	799	763	794	735
Digestible Organic Matter in the Dry matter (g/kg DM)	736	767	749	734	703	726	653

Table 5 shows the degradability constants (a, b and c) and the ED values calculated. The ED of both CP and DM for grazed grass is high (~89% and ~75% respectively). The ED for DM decreased between April and July but increased again from August to September. The lowest value in July (72%) was associated with the highest b fraction. The ED for CP followed the same pattern in line with the CP concentration in the samples.

Table 5. Degradability constants (a, b and c) and calculated effective degradabilities (ED) (%) of grass

Grass		a	b	c	ED
April	DM	61.6	37.2	0.06	78.6
	CP	85.0	14.1	0.07	92.1
May	DM	62.9	37.1	0.04	76.5
	CP	81.1	18.2	0.05	88.5
June	DM	56.1	40.1	0.05	74.6
	CP	76.9	19.6	0.10	88.7
July	DM	54.7	44.5	0.04	71.7
	CP	77.3	21.5	0.05	86.7
August	DM	52.9	38.9	0.06	72.7
	CP	75.4	20.0	0.10	87.9
September	DM	57.1	38.5	0.05	74.5
	CP	78.2	18.3	0.08	88.5
October	DM	59.0	33.7	0.09	78.5

	CP	82.3	14.3	0.18	92.8

The PDIN values for grass were lowest in May, June and July (108, 114 and 110 g/kg DM, respectively) which reflected the lower crude protein content of the grass at these times (166, 176 and 169 g/kg DM). The PDIE values for grass tended to be relatively constant throughout the grazing season and they did not have the same level of variability as in the PDIN values (Table 6). The lowest value was obtained in October, which coincides with the lowest digestibility. The PDIME content remained relatively the same throughout the major part of the season, which is due to the relatively constant supply of fermentable organic matter (FOM) per kg of grass DM.

Table 6. The [1]PDI values (g/kg DM) of the grazed grass samples incubated in the rumen for the estimation of degradability

	April	May	June	July	August	September	October
PDIA	14	15	16	17	18	18	14
PDIME	66	69	67	66	63	65	59
PDIMN	129	93	98	93	105	114	147
PDIE	80	84	83	83	81	83	73
PDIN	143	108	114	110	123	132	161

[1] See Table 3 for definitions

As for grass silage the relationship between the PDI values and the chemical composition of grazed grass was determined by regression analysis. When the parameters CP, DMD, OMD, ash and NDF were included in the regression model for grazed grass, equations for the prediction of PDIE ($R^2 = 0.987$) (equation 4) and PDIN ($R^2 = 1.000$) (equation 5) were as follows:

Equation 4. PDIE = 181.4 - 0.104 (NDF) - 0.195 (ash) - 0.047 (OMD)

Equation 5. PDIN = 35.7 + 0.634 (CP) - 0.026 (ash) - 0.027 (OMD) - 0.021 (NDF)

Regressing PDIN on CP only, resulted in the following simple equation for grazed grass ($R^2 = 0.999$) (equation 6):

Equation 6. PDIN = 3.79 + 0.629 (CP)

The regression equations for both grass silage and grazed grass are important practical outcomes from this study in that they provide simple relationships that allow the prediction of PDIE and PDIN from chemical analysis normally available. It would be desirable to subject further samples to degradability and chemical analysis so that confidence in such equations is increased.

PDI values of the concentrate ingredients

The mean CP and PDI values for the concentrate ingredients are shown in Table 7. Five samples of each ingredient were analysed and generally there was substantial variation in chemical composition between the samples. For this reason, the PDI values tend to have large standard deviations.

Of the energy ingredients (barley, beet pulp, palm kernel and pollard) pollard had the highest CP content but palm kernel meal had the highest PDIN content due to fact that palm kernel meal had a very high level of PDIA in contrast to barley, pollard and beet pulp. The relatively lower PDIN in barley and beet pulp was associated with these feeds having a lower CP content compared to palm kernel meal or pollard.

Of the moderate protein ingredients (copra meal, maize distillers, maize gluten feed and malt sprouts) maize distillers grains had the highest PDIN which is a reflection of the high CP (g/kg DM) content. The PDIN was composed mainly of a large PDIA value and a low PDIMN value, which was similar for copra meal.

Of the high protein ingredients (cottonseed meal, rapeseed meal, soyabean meal and sunflower meal) soyabean and cottonseed meals had the highest CP content while rapeseed meal and sunflower meals were slightly lower. PDIN reflected the CP contents of the feeds and all samples were high in PDIN. Both cottonseed and soyabean meals had a high concentration of PDIMN compared to PDIME. Sunflower meal had low values for PDIMN and PDIME due to a low ED of CP and a low digestibility indicating a low level of FOM.

Table 7. The mean (s.d. in parentheses) [1]CP and [1]PDI values (g/kg DM) for the high protein feeds

	CP	[2]PDIA	PDIME	PDIMN	PDIE	PDIN
Barley	112 (12.2)	17 (4.1)	72 (1.6)	59 (8.2)	89 (4.5)	76 (7.9)
Beet Pulp	105 (7.0)	34 (10.0)	70 (2.2)	38 (10.3)	104 (8.8)	72 (4.3)
Palm Kernel	178 (30.0)	109 (31.1)	53 (2.8)	95 (2.8)	81 (4.3)	123 10.1)

Pollard	182 (15.1)	29 (2.0)	53 (2.8)	95 (2.8)	81 (4.3)	123 (10.1)
Copra meal	227 (4.1)	111 (15.5)	49 (1.8)	64 (8.6)	160 (14.1)	175 (6.9)
Maize Distillers	301 (14.5)	170 (36.6)	36 (2.6)	60 (26.3)	206 (36.4)	230 (14.5)
Maize Gluten	239 (12.4)	62 (14.0)	53 (2.8)	109 (14.7)	115 (13.7)	171 (7.2)
Malt Sprouts	199 (18.1)	61 (4.6)	47 (3.1)	82 (9.8)	108 (2.8)	143 (12.3)
Cottonseed Meal	421 (55.9)	99 (18.7)	46 (4.7)	195 (34.1)	145 (15.7)	295 (37.3)
Rapeseed meal	391 (19.4)	124 (28.1)	46 (6.6)	158 (9.9)	170 (21.8)	282 (19.8)
Soyabean Meal	524 (17.6)	155 (26.2)	61 (2.7)	233 (18.3)	216 (23.6)	387 (15.9)
Sunflower Meal	314 (42.5)	154 (27.3)	33 (3.9)	95 (12.0)	187 (26.9)	249 (35.2)

[1] CP = crude protein [2] See Table 4 for definitions

Conclusion

This study produced PDIE and PDIN values for Irish grass silages and pasture. These are the first Irish values to be actually determined for use in the new Protein system. The PDIE and PDIN values of grass silages were variable but could be predicted with reasonable confidence from commonly available chemical analysis.

The PDIN content of grazed grass increased from early to late in the season as a result of increased CP content, while there was a small decrease in PDIE in line with the small decrease in digestibility of the grass. From the regression equations developed the PDIE and PDIN content of grazed grass could also be predicted with reasonable confidence from commonly available chemical analysis. A further study determining the PDIE and PDIN of a larger number of samples would be worthwhile.

A lot of variation existed in the PDI values of concentrate ingredients, both between similar ingredients (i.e. soyabean meal, rapeseed meal, etc.) and within samples of the same ingredient, mainly as a result of the variable chemical composition and CP degradability of the different batches available on the Irish market.

Experiment 2. The Response to and Effect of Balancing PDIE and PDIN in the Diet on Milk Production and N Excretion by Dairy Cows

Introduction

Responses in production to protein supplementation are variable with both positive and neutral responses being reported. A large proportion of the response if obtained can be explained by factors other than direct protein supply such as increased dry matter and energy intake.

Studies have shown that between 64-85% of the N ingested by dairy cows is excreted in the faeces and urine. Nitrogen excretion is related to the protein content and degradability of the forage and concentrate supplement in the diet. Efficiency of N use is an important factor in supplementation of protein in the diet, as a higher efficiency would reduce production costs and also reduce the loading of waste nutrients on the environment.

Utilisation of dietary N is affected by the energy content of the diet. The correct balance of energy and N is required for the production of microbial protein which accounts for 600-700 g/kg of the AA that are absorbed in the small intestine. The French protein (PDI) system can be used to balance the energy and N in the diet. In this system the protein value of the feeds and the animal requirements are both expressed in terms of the true protein truly digestible in the small intestine, abbreviated as PDI.

The objective of this study was to evaluate the response to PDIE and PDIN in the diet of dairy cows and to assess the effect of better balancing the PDIE and PDIN supply on the efficiency of N utilisation for milk protein production and on N excretion.

Materials and methods

Experiment design and animals

Twenty autumn calving Holstein-Friesian dairy cows with an average lactation number of 4.7 (range 2-5) were blocked into five squares according to calving date and mean milk yield for the 2 weeks before the start of the experiment. Mean calving date was October 1st (range Sept 26th to Oct 8th) and the cows were on average 55 days in milk at the beginning of the experiment.

The experiment was a balanced Latin Square design with four treatments and four periods of four weeks duration each. Each of the four treatments consisted of a concentrate, maize silage and grass silage in the proportions 37:38:25 on a DM basis. The forages were mixed in a diet feeder (Keenan, Borris, Co. Carlow) and were offered once daily at 0900h on an ad-libitum basis with concentrate being offered at a rate of 370 g/kg of the previous day’s total DMI in three equal parts at 0830h, 1230h, and 1630h. Forage refusals were weighed back every morning at 0830h.

Experimental treatments and diets

The diets were designed to have the following PDIE and PDIN levels relative to requirements, based on a daily intake of 20.8 kg DM and a milk yield of 35 kg.

Diet A - 0.88 of PDIE requirements (91 g/kg DM) and 1.02 of PDIN requirements (105 g/kg DM)

Diet B - 0.99 of PDIE requirements (103 g/kg DM) and 1.06 of PDIN requirements (110 g/kg DM)

Diets C - 1.02 of PDIE requirements (106 g/kg DM) and 1.18 of PDIN requirements (123 g/kg DM)

Diet D - 1.02 of PDIE requirements (106 g/kg DM) and 1.37 of PDIN requirements (142 g/kg DM)

The adjustments in PDIE and PDIN supplies were achieved by feeding a concentrate with a different ingredient formulation on each treatment (Table 8).

Table 8. Ingredient (g/kg) and chemical composition (g/kg DM unless stated otherwise) of the four concentrates

	Concentrate A	Concentrate B	Concentrate C	Concentrate D
Barley	240	257	231	100
Unmolassed beet pulp	-	222	-	-
Maize gluten feed	313		100	177
Soya bean meal	107	386	378	366
Rapeseed meal	200	-	161	200
Molasses	50	50	50	50
Urea	10	-	-	27

Megalac	50	50	50	50
Min/vit	30	35	30	30
Dry matter (g/kg)	875	899	860	850
Crude protein	268	281	328	402
Crude fibre	73	58	57	64
UFL/kg	0.97	1.03	1.01	0.97
PDIN	193	207	249	296
PDIE	137	164	188	185

Samples and Animal Measurements

Milk yield was recorded automatically on a daily basis. Milk composition (fat, protein, and lactose) was determined twice weekly (Tuesday and Thursday) during weeks 3 and 4 of each period from successive morning and evening milk samples. Cow body weight was measured on Thursday of each week after morning milking. Body condition score (BCS) on a scale of 1 (thinnest) to 5 (fattest) of the cows was determined at the beginning and end of each four week experimental period. Blood samples were taken, into lithium heparin vacutainers, from the coccygeal vessels in the morning and evening, after milking but before feeding, on the Friday of weeks 3 and 4 of each period. The metabolites analysed were glucose, non-esterified fatty acids (NEFA), ß-hydroxy butyrate (ßHB), urea and total protein. Samples of urine and faeces were taken separately for 5d (Monday to Friday; morning and evening) in week 4 of each period. These samples were frozen immediately and before analysis were thawed and composited for the five collection days to give a representative sample for each cow for each period. Samples were used to determine faecal N, and urinary N. Rumen degradability of the crude protein in the concentrates, maize silage and grass silage was determined and the PDIE and PDIN values calculated as in experiment 1.

Calculation of the N balance per day

The N supply and excretion was determined by using the equations below.

N intake (g/d) = (CP of diet (g/kg) * DMI) / 6.25

Faecal N (g/d) = ((1- DM digestibility of the diet) * DMI) * faecal N (g/kg DM)

Milk N (g/d) = Yield of milk protein (g/d) / 6.38

Endogenous losses = 2.4 * DMI (Vérité and Peyraud, 1989)

Scurf and hair loss = (((3.25 * body weight $^{0.75}$)/ 6.25)- endogenous loss)

Urine N (g/d) = N intake – faecal N – milk N – scurf and hair loss

Data were analysed by the PROC GLM procedure of SAS using a model, which included treatment, period, square, cow within square and square by treatment. This was applied to DMI, milk yield, milk composition, PDI and UFL intake and N utilisation. Mean data from weeks three and four of each four-week period was used to compare the treatments.

Results

The grass and maize silages offered were of good quality. The grass silage had a mean DM of 213 g/kg, pH of 3.68, crude protein of 130 g/kg DM, PDIE of 73 g/kg DM, PDIN of 84 g/kg DM and *in-vitro* DMD of 762 g/kg. The corresponding values for the maize silage were 285 g/kg, 3.76, 91 g/kg DM, 60 g/kg DM, 60 g/kg DM, 685 g/kg and a starch content of 248 g/kg DM.

Effect of diet on intake and milk production

Dry matter intake was significantly lower on diet B compared to the other three and it was significantly higher on diet D compared to A and B (Table 9). UFL intakes were higher on diets C and D compared to A and B. Generally PDIE and PDIN intakes were higher than estimated because DM intake was between 11 and 18% higher than that used in making the estimates. PDIE intake increased significantly from A to B to C and diet D was not different from C. PDIN intake was not different between diets A and B but it increased significantly going to diet C and again to diet D. PDI balance (PDIN intake – PDIE intake) was lowest on diet B and increased significantly between diets in the order B<A<C<D.

Table 9. The effect of dietary treatments on DM, UFL, CP, PDIN and PDIE intake per day and PDI balance

	DIET				
	A	B	C	D	sed
DM (kg/d)	23.7 [a]	23.0 [b]	23.9 [ac]	24.6 [c]	0.20

UFL (/d)	20.8 [a]	20.7 [a]	21.4 [bc]	21.6 [c]	0.18
CP (g/d)	3945 [a]	3960 [a]	4512 [b]	5293 [c]	37.45
PDIE (g/d)	2185 [a]	2362 [b]	2656 [c]	2695 [c]	20.20
PDIN (g/d)	2746 [a]	2799 [a]	3264 [b]	3767 [c]	26.68
PDI balance (g/kg) *	561 [a]	437 [b]	608 [c]	1073 [d]	7.47

Means within rows having different superscripts differ significantly (P<0.05)

* PDI balance = PDIN intake/day – PDIE intake/day

Diet A had a lower milk yield than the other three diets, being significantly lower (P< 0.05) than C and D (Table 10). There were no significant differences between diets B, C and D in milk composition except for a significantly higher milk protein yield on diet D than B (P<0.05). Diet A had a numerically lower protein yield than diets B and C and a significantly lower protein yield than diet D (P<0.05). Diet A had a higher protein concentration than diet B (P<0.05). There was no significant difference between treatments in lactose concentration but cows on diet A had a lower (P<0.05) lactose yield compared to C to D.

Table 10. The effect of dietary treatments on milk yield and composition

	DIET				
	A	B	C	D	sed
Milk Yield (kg/d)	34.3 [a]	35.1 [ab]	35.9 [b]	35.8 [b]	0.34
Milk fat (kg/d)	1.33	1.36	1.36	1.36	0.02
Milk protein (kg/d)	1.04 [a]	1.05 [a]	1.07 [ab]	1.08 [b]	0.01
Milk lactose (kg/d)	1.61 [a]	1.65 [ab]	1.67 [b]	1.66 [b]	0.02

Milk fat (g/kg)	38.8	38.9	38.2	38.3	0.05
Milk protein (g/kg)	30.4^{a}	29.9^{b}	30.1^{ab}	30.2^{ab}	0.01
Milk lactose (g/kg)	47.5	46.7	47.8	48.5	0.09

Means within rows having different subscripts differ significantly (P<0.05)

Effect of diet on N utilisation and microbial protein production

There was an increase in N intake between diets A and D with a significant increase (P<0.05) between diets B and C and C and D (Table 11). Diet B resulted in significantly less faecal N than the other three diets. There was no significant difference between the diets in the amount of milk N produced. Urine N excretion increased significantly between diets in the order A<B<C<D.

Table 11. The effect of diet on N intake and N output in milk faeces and urine

		DIET			
	A	B	C	D	sed
N intake (g/d)	620.1^{a}	623.4^{a}	723.8^{b}	833.7^{c}	7.34
Faecal N (g/d)	146.0^{a}	130.0^{b}	145.4^{a}	144.8^{a}	2.81
Milk N (g/d)	166.1	166.0	166.6	171.9	2.71
Urine N (g/d)	301.0^{a}	317.9^{b}	394.3^{c}	488.6^{d}	6.09

Means within rows having different superscripts differ significantly (P<0.05)

Effect of diet on the efficiency of N utilisation and milk production

Less milk was produced per kg DMI in both diets A and D than in diets B and C, which had similar values (Table 12). Milk net energy (NE) output per kg DMI followed the same pattern.

Diets A and B were significantly more efficient in the conversion of dietary N to milk N compared to diets C and D.

Table 12. The effect of dietary treatments on the efficiency of milk production and N utilisation

		DIET			
	A	B	C	D	sed
Efficiency of milk production					
Milk yield/ DMI (kg/kg)	1.45 [a]	1.54 [b]	1.52 [b]	1.46 [a]	0.013
Milk NE /kg DMI (UFL/kg)	0.63 [a]	0.67 [b]	0.66 [bc]	0.64 [ac]	0.008
Efficiency of N utilisation					
Total N excretion / N intake (g/g)	0.72 [a]	0.72 [a]	0.75 [b]	0.78 [c]	0.01
Milk N / N intake (g/g)	0.27 [a]	0.27 [a]	0.24 [b]	0.21 [c]	0.002

Means within rows having different superscripts differ significantly (P<0.05)

The mean ßHB was lower on diet A compared to the other three diets (P< 0.01) (Table 13). There were no significant differences between diets in plasma protein, glucose or NEFA concentrations. Plasma urea was significantly increased between diets in the order A<B<C<D (P< 0.001).

Table 13. The effect of dietary treatments on plasma metabolite concentrations (mmol/l unless stated otherwise).					
		DIET			
	A	B	C	D	sed
ß hydroxy butyrate	0.64 [a]	0.77 [b]	0.76 [b]	0.77 [b]	0.026
Glucose	4.21	4.15	4.19	4.17	0.025
Non-esterified fatty acids	0.09	0.11	0.10	0.09	0.013
Protein (g/l)	83.7	83.8	84.5	84.3	0.502
Urea	5.19 [a]	6.08 [b]	6.63 [c]	10.67 [d]	0.105

Means within rows having different superscripts differ significantly ($P<0.05$)

Conclusions

The current recommended levels of protein supply for dairy cows need to be revised, with proper balancing of protein and energy in the diet using the French protein (PDI) system. Results from this study suggest that a concentration of approximately 170g CP/kg DM is sufficient for dairy cows producing 35 kg/day of milk with 30 g/kg of protein under Irish conditions. The optimum concentration of dietary PDI is approximately 103 g/kg DM which was equivalent to the concentration of PDIE in diet B. Increasing dietary PDIN concentration did not result in greater milk or protein output, mainly due to reduced efficiency of DM and N utilisation. However, increasing dietary PDIN did result in increased excretion of N to the environment. The efficiency of N use and minimising N excretion must also be objectives in a sustainable feeding regime and these can be achieved by having the correct balance of PDIE and PDIN in the diet. There was no apparent loss in production efficiency at a dietary CP concentration of 170 g/kg DM, with a good balance between PDIN and PDIE, compared to dietary CP concentrations of 189 or 215 g/kg DM.

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