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Validation of protein evaluation systems by means of milk production experiments with dairy cows

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Abstract

Protein evaluation systems (CP, DCP, PDI, AAT, AP, MP, AAS and DVE) were validated using data from 15 production experiments with dairy cows, carried out in The Netherlands. Only treatments that were deficient in protein according to at least one system were selected. Average production was 31.2 kg fat and protein corrected milk d^{-1} and 989 g milk protein d^{-1}. The observed milk protein production was compared with milk protein production predicted from the protein supply and requirements in each system.
The difference between observed and predicted milk protein production expressed as the absolute and relative prediction error was smallest for the DVE-system (–2 g d^{-1}; respectively 5.7%) and increased in the order CP (–22 g d^{-1}; 6.7%), PDI (–19 g d^{-1}; 7.8%), DCP (–44 g d^{-1}; 8.8%), AP (–37 g d^{-1}; 9.3%), AAS (100 g d^{-1}; 11.7%), AAT (112 g d^{-1}; 13.4%) and MP-system (204 g d^{-1}; 22.9%). Predictions can be improved when a variable efficiency of milk protein production is used. In the DVE-system the observed efficiency decreased with increasing protein to energy ratio in the diet and milk production level. It was concluded that under Dutch conditions the prediction of milk protein production decreased in the order DVE, CP, PDI, DCP, AP, AAS, AAT and MP-system.

Keywords: dairy cows, protein, evaluation systems

Introduction

Protein evaluation for ruminants had until recently in many countries been based on digestible crude protein (DCP). Although the system was satisfactorily used, shortcomings became more and more apparent. A major disadvantage of the DCP-system is that it does not take into account protein degradation and microbial protein synthesis in the rumen. With the DCP-system it is therefore not possible to use recently acquired knowledge of the protein digestion process to improve efficiency of N utilisation in dairy cows. During the last decades new protein evaluation systems for ruminants, describing the protein digestion process more detailed than the DCP-system, have been developed and introduced (Van der Honing and Alderman, 1988).

The protein unit (PU) of a feedstuff and the requirements of PU in these systems are expressed as true protein absorbable from the small intestine. Examples are: PDI (Vérité et al., 1987), AAT (Madsen, 1985), AP (NRC, 1985), MP (AFRC, 1992), AAS (Ausschüss für Bedarfsnormen, 1986) and DVE (Tamminga et al., 1994) (for explanation of abbreviations of systems see Table 2). All these systems use a factorial approach in which PU originates from feed protein escaping rumen degradation (escape protein) and from microbial protein synthesized in the rumen. The PU requirement is separated into requirements for milk production, maintenance, gestation and, in case of the MP and DVE-system, retention or mobilisation of body reserves. Because systems use different factors and tables to calculate PU supply and requirements, the optimal crude protein (CP) concentration and escape protein fraction in the diet vary considerably between systems (Waldo and Glenn, 1984; Alderman, 1987).

Although numerous experiments were carried out to quantify the effect of protein feeding or post-ruminal infusion on milk protein production, only a limited number of these data were used to directly validate protein evaluation systems (Jarrige and Alderman, 1987). Generally PU intake in the newly developed systems showed a better relationship with milk protein production than CP or DCP intake (Thuen and Vik-Mo, 1985; Vik-Mo, 1985; Bricenco et al., 1988). The difference between predicted and observed milk protein production varied between systems and experiments (Vik-Mo, 1985; MacRae et al., 1988; Sloan et al., 1988; Garnsworthy, 1989; Cody et al., 1990; Broderick et al., 1990; Robinson et al., 1991; Susmel et al., 1991). This variation could be due to differences in experimental design, type of feedstuff and production level and type of animals used.

The objective of the present study was to validate protein evaluation systems by comparing the predicted to the observed milk protein production, measured in production experiments carried out in The Netherlands with high yielding dairy cows having a PU intake below PU requirements in at least one system.

Material and methods

Production experiments

Data from 15 production experiments with early to mid-lactating dairy cows were available. The experiments were carried out at four different locations. Information on the number of animals, treatments, experimental weeks and type of diets is given in Table 1. In the experiments Dutch Friesian and Holstein Friesian cows were used which were housed in a loose housing or tie stall and fed individually. Roughage

Table 1. Number of animals, treatments and duration of the experiments.

Experiment nb.	ref[a]	Number of animals	Roughage type[b]	Treatments: Description[c]	Total	Number Selected[d]	Roughage: Concentrate	Weeks[e] PP	MP
1	1	59	GS	%ECP	4	0	45:55	3	21
2	2	28	GS	CP	2	0	50:50	3	17
3	2	52	GS,MS	%ECP,GS:MS-ratio	8	0	40:60; 44:56	3	15
4	3	28	G,MS	G:MS-ratio,feeding method	4	1	80:20	2	6
5	3	24	G,MS	G substitution	4	3	80:20; 60:40	2	6
6	4	92	GS	%ECP	4	2	45:55	4	16
7	4	120	GS,MS	%ECP,CP,GS:MS ratio	8	6	50:50; 35:65	5	12
8	4	102	GS,MS	%ECP	6	1	45:55	1	17
9	4	72	GS,MS	%ECP	4	1	45:55	4-5	12
10	4	98	GS,MS	%ECP,CP	8	4	50:50; 55:45	5	12
11	2	60	GS,MS	DVE, GS:MS ratio	5	2	50:50	3	13
12	4	47	GS,MS	DVE	2	2	50:50	1	6
13	5	36	GS,MS	DVE	3	2	35:65	4	7
14	6	44	MS	DVE	4	4	65:35	3	20
15	6	43	MS	DVE	4	2	65:35	3	16
Total		905			70	30			

[a] Reference: 1: Rijpkema et al. (1990), 2: Rijpkema et al. (unpublished from IVVO-DLO, Lelystad), 3: Valk et al. (1990), 4: Veen et al. (unpublished from CLO-Institute for Animal Nutrition 'De Schothorst', Lelystad), 5: Boxem et al. (unpublished from Research Station for Cattle, Sheep and Horse Husbandry, Lelystad); 6: Hof et al. (1994)
[b] GS = grass silage, MS = maize silage, G = fresh grass;
[c] %ECP = escape protein fraction, CP = CP level, DVE = DVE level;
[d] Treatments with PU supply in at least one system below PU requirements;
[e] PP = pre-period, MP = main-period. The pre-period started directly after calving, except for experiment 4 (in lactation week 8) and experiment 5 (in week 14).

consisted of fresh grass, grass silage, maize silage or a mixture of these roughages (Table 1). In each experiment roughage intake, concentrate intake, milk production, milk protein and fat content and body weight of each cow were measured weekly.

Calculation of the energy and PU supply and requirement

Contents of dry matter (DM), organic matter (OM), CP, and Dutch Net Energy Lactation (NEL) were obtained from laboratory analysis of individual roughages, concentrates and concentrate ingredients. Lacking values were taken from the Dutch Feeding Tables (CVB, 1991). The fat content of grass, grass silage and maize silage was assumed to be 40, 40 and 30 g kg^{-1} DM, respectively.

The energy supply and requirements were calculated according to the Dutch NEL-system (Van Es, 1975, 1978). The following protein evaluation systems were used in the validation: CP and DCP-system (CVB, 1990), PDI-system (Vérité et al., 1987), AAT-system (Madsen, 1985), AP-system (NRC, 1985), MP-system (AFRC, 1992), AAS-system (Ausschüss für Bedarfsnormen, 1986) and DVE-system (Tamminga et al., 1994). The coefficients and factors used in each system to calculate PU supply and requirements are summarised in Table 2. In the AAS and DVE-system the sum of escape and microbial PU is diminished with respectively endogenous duodenal AAS and metabolic faecal DVE.

The protein escape fraction and digestibility of escape protein of the diets were calculated on the basis of the individual feedstuffs. Values for individual feedstuffs were taken from the tables of each system (AFRC, 1992; CVB, 1991; Hvelplund, 1985; Jarrige, 1990; Madsen and Hvelplund, 1985; NRC, 1988). When values were not available in those tables, data were obtained from the Dutch Feeding Tables (CVB, 1991), based on studies of Van Straalen and Tamminga (1990). The content of silage fermentation products in the PDI-system was taken from Jarrige (1990).

Potential microbial CP synthesis was calculated based on the rumen available energy and on the rumen degraded CP, both multiplied with the respective efficiencies, and for the AAT-system including recycling of degraded CP (Table 2). The lowest value in the diet was used as the actual microbial CP synthesis. The difference between microbial CP based on CP and based on energy was called the rumen protein balance (RPB).

For each system the PU available for milk production was calculated from total PU supply minus PU requirement for maintenance and for the MP and DVE-system also for retention or mobilisation of body protein. Maintenance requirements in the DVE-system only consist of requirements for endogenous urinary-N and skin-N, because metabolic faecal requirements were already substracted from the DVE supply. Milk protein production was predicted from the PU available for milk production and the efficiency of milk protein production. The absolute prediction error of milk protein production (APE) was calculated as the difference between predicted and observed milk protein production. NEL and PU supply, PU requirements and APE were calculated per cow and lactation week. Feed intake, milk production, NEL and PU supply and requirements and APE were averaged per treatment, resulting in 70 observations for further calculations.

Validation and statistical analysis

The validation of protein evaluation systems was carried out using a restricted database in which only treatments were included in which PU supply may have been limiting for milk protein production. Selected treatments therefore had to meet the condition that total PU supply was in at least one system below total PU requirements. This restricted the database to 30 treatments (Table 1), of which respectively 21 treatments were deficient in CP, 23 in DCP, 18 in PDI, 1 in AAT, 19 in AP, 0 in MP, 1 in AAS, and 16 in DVE. Differences between systems in PU supply, PU requirements and APE were tested using analysis of variance.

Validation of the systems was based on the following criteria:

1. The relationship between PU (and NEL) intake and milk protein production, using regression analysis;
2. The difference between predicted and observed milk protein production for each system, calculated as the mean square prediction error (MSPE), according to Bibby and Toutenberg (1977):

$$MSPE = \sum_{i=1}^{n} (O_i - P_i)^2/n$$

in which O_i and P_i is the observed and predicted milk protein production, and n is the number of observations (30). The MSPE was decomposed into the error due to overall bias (intercept different from 0), the error due to deviations of the regression slope from 1 and the error due to disturbances (Bibby and Toutenburg, 1977). The root of the MSPE expressed as percentage of the observed mean is used as measure of the relative prediction error (RPE).

The relationships between APE and feed intake variables (RPB, escape PU, microbial PU, NEL, NEL/PU for milk production), were studied by calculating correlation coefficients. These relationships were used to identify possible causes of the prediction error.

Statistical analysis was carried out using the Genstat Statistical Program (Genstat 5 Committee, 1987). Differences were declared at $p < 0.05$, unless stated otherwise.

Results

Milk production, feed intake, PU supply and PU requirements

Live weight, milk production and intake of DM, NEL and PU of the selected observations are summarized in Table 3. Milk protein production was on average 989 g d^{-1} and ranged from 812 to 1271 g d^{-1}. PU intake in the new systems was highest for the AP-system (65% of CP) and decreased in the following order: MP (63% of CP), AAS and PDI (62% of CP), AAT (61% of CP) and DVE-system (54% of CP).

PU supply from escape protein was different between systems, except for the PDI and AP-system, and varied from 21% of the sum of escape and microbial PU supply in the AAS to 46% in the DVE-system (Table 4). Microbial PU supply in the AAS-

Table 2. Summary of the factors and coefficients used in the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system.

	System[a]							
	CP	DCP	PDI	AAT	AP	MP	AAS	DVE
Protein Unit requirements ($g\ d^{-1}$)								
– Lactation								
– efficiency	0.38	0.52	0.64	0.73	0.65	0.72[b]	0.80	0.64
– Maintenance								
– metabolic faecal	–	–	–	–	$0.09*IDM$[c]	$1.43*W^{0.75}$	$18.19*DM$	[d]
– endogenous urine	–	–	–	–	$4.10*W^{0.5}$	$0.75*W^{0.75}$	[e]	$4.10*W^{0.5}$
– scurf	–	–	–	–	$0.30*W^{0.6}$	$0.11*W^{0.75}$	$0.11*W^{0.75}$	$0.30*W^{0.6}$
– total	[f]	[g]	$3.25*W^{0.75}$	$3.30*W^{0.75}$	–	–	–	–
– Body protein	–	–	–	–	–	[h]	–	[h]
Protein Unit supply ($g\ kg^{-1}$ DM)								
Total	Table	Table						
– Microbial (M)								
– Energy unit	–	–	MOF[i]	DCH[j]	TDN[k]	FME[l]	ME[l]	FOM[m]
– MCP/energy unit	–	–	145	179	[n]	11	11.92	150
– MCP/degraded CP	–	–	0.90	1.00[j]	0.90	1.00	1.20	1.00
– M true protein/MCP	–	–	0.80	0.70	0.80	0.75	0.70	0.75
– MPU/M true protein	–	–	0.80	0.85	0.80	0.85	0.90	0.85
– Escape (E)								
– escape protein/CP	–	–	Table	Table	Table	Table	Table	Table[o]
– correction factor	–	–	1.11	–	–	–	–	1.11
– E true protein/ECP	–	–	1.00	(0.65/0.85[p])	1.00	1.00	0.70	1.00
– EPU/E true protein	–	–	Table	0.82	0.80	Table	0.90	Table
– Correction factor	–	–	–	–	–	–	$-15*DM$[q]	$-0.075*IDM$[c,d]

[a] CP = Crude Protein; DCP = Digestible Crude Protein (CVB, 1990); PDI = Protein Digestible dans l'Intestin (Vérité, et al., 1987); AAT = Aminosyres Absorbered fra Tyndtarmen (NKJ-NJF, 1985); AP = Absorbable Protein (NRC, 1985); MP = Metabolisable Protein (AFRC, 1992); AAS = Absorbierbare Amino Säure (Auschüss für Bedarfsnormen, 1986); DVE = DarmVerteerbaar Eiwit (Tamminga et al., 1994).

[b] 0.72 = 0.68 (efficiency of true milk protein synthesis) / 0.95 (true protein content in milk CP)

[c] IDM (indigestible dry matter) = DM - digestible OM (DOM) - digestible ASH

[d] DVE-supply is corrected for metabolic faecal protein excretion

[e] Endogenous urinary requirement for AAS: $(37.0 * {}^{10}\log W - 42.2)$

[f] Maintenance requirement for CP = (0.43*W + 130 - (600-W)*0.5)/0.7

[g] Maintenance requirement for DCP = 0.43*W + 130 - (600-W)*0.5

[h] The PU requirement in the MP and DVE requirement for growth or supply from mobilisation of body reserves is dependent on energy balance:
for growth: 35 g DVE or 30 g MP/Mcal NEL positive energy balance
for mobilisation: 27 g DVE or 22 g MP/Mcal NEL negative energy balance

[i] MOF (fermentable organic matter, PDI-system) = DOM - escape CP - crude fat - fermentation products

[j] DCH (total digestible carbohydrates) = digestible crude fibre (DCF) + digestible non-fatty-extracts (DNFE); 200 g rumen degraded CP d^{-1} can be recycled to the rumen

[k] TDN (total digestible nutrients) = DOM + 1.25*digestible crude fat (DFAT)

[l] ME = metabolic energy calculated according to CVB (1991),
FME = fermentable ME = ME - 35 * crude fat - 0.1 * ME (for silages) and ME - 35 * crude fat (for other feedstuffs)

[m] FOM (fermentable organic matter, DVE-system) = DOM - escape CP - crude fat - fermentation products * 0.5 - escape starch;

[n] Microbial crude protein = (-31.86 + 26.12*(TDN/1000)) * 6.25

[o] Escape CP/CP for grass, grass and grass silage is predicted from the CP and DM content and the number of days elapsed since the first of April (Tamminga et al., 1994)

[p] True protein/CP roughages = 0.65; concentrates = 0.85

[q] Correction for endogenous protein flow to the duodenum

Table 3. Average, minimum, maximum and standard deviation (Std.) of live weight, milk production and DM, net energy lactation (NEL) and protein unit (PU)-intake (n=30).

	Average		Minimum		Maximum		Std.
Live weight (kg)	589		542		645		30
Milk production							
– FPCM[a] (kg d^{-1})	31.2		25.1		41.1		4.7
– protein (g kg^{-1})	32.3		30.4		34.4		1.0
– protein (g d^{-1})	989		812		1271		130
– fat (g kg^{-1})	41.9		31.8		48.0		3.8
– fat (g d^{-1})	1295		914		1747		236
DM-intake (kg d^{-1})							
– total	20.0		16.8		24.0		2.3
– roughage	10.9		5.9		16.2		2.9
– concentrate	9.0		3.4		11.6		2.5
– roughage (g kg^{-1} DM)	545		339		822		126
NEL-intake (MJ d^{-1})	133.3		107.0		164.7		18.4
PU intake (g d^{-1}) (between brackets: % of requirements)							
– CP	3084	(98)	2611	(90)	3871	(111)	340
– DCP	2195	(96)	1802	(85)	2772	(116)	248
– PDI	1904	(98)	1560	(84)	2425	(109)	240
– AAT	1886	(108)	1557	(99)	2382	(117)	231
– AP	2006	(97)	1678	(85)	2541	(108)	225
– MP	1952	(113)	1563	(103)	2419	(130)	216
– AAS	1907	(107)	1622	(98)	2410	(112)	219
– DVE	1664	(100)	1359	(89)	2077	(108)	240

[a] FPCM = fat and protein corrected milk
= [0.337 + 0.116*fat content (%) + 0.06*protein content (%)] * milk production (kg d^{-1})

system was highest compared to the other systems, for both methods of calculating microbial PU: from rumen available energy and from degraded CP. In the PDI-system microbial PU supply was lowest for both calculation methods. In the AP and MP-system, microbial PU supply from rumen degraded CP was lower than from rumen available energy, resulting in high negative RPB for those systems. The actual microbial PU supply was highest for the AAS-system, and showed a small, although significant, variation among the other systems. The RPB was negative (microbial CP synthesis limited by rumen available protein) in 11, 12, 30, 25, 9 and 9 treatments of the 30 treatments for the PDI, AAT, AP, MP, AAS and DVE-system respectively.

PU requirements for maintenance were different between systems, except for the PDI and AAT-system, and the AP and AAS-system, and varied from 7% of the PU intake in the DVE-system to 29% in the AAS-system. The energy balance varied between –26 and 27 MJ NEL d^{-1} with an average of 0.4 MJ NEL d^{-1}. The correction for retention or mobilisation of body protein calculated from the NEL balance ranged from –152 to 207 g d^{-1} in the MP-system and –190 to 244 g d^{-1} in the DVE-system. The average was about 10 g MP or DVE d^{-1} which is 0.5% of the total MP or DVE requirement (Table 4). The PU available for milk production was highest in the MP-system. In the AAS-system, PU available for milk production was lowest com-

Table 4. Average protein units (PU) supply and requirement, and predicted milk protein production in the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system.

System[1]	PU-supply						PU-requirement		PU available for milk	Predicted milk protein production
		Microbial[3]								
	Escape[2]	Energy	CP	Actual	Total	RPB[4]	Maintenance	Body		
CP	–	–	–	–	3084^{a}	–	539^{a}	–	2545^{a}	967
DCP	–	–	–	–	2195^{b}	–	377^{c}	–	1818^{b}	945
PDI	851^{b}	1103^{e}	1145^{e}	1053^{d}	1904de	66^{c}	388^{b}	–	1516de	970
AAT	713^{d}	1240^{d}	1275^{b}	1173^{b}	1886^{e}	60^{c}	394^{b}	–	1492de	1101
AP	820^{b}	1456^{b}	1186^{c}	1186^{b}	2006^{c}	−422^{a}	541^{a}	–	1465^{e}	952
MP	778^{c}	1330^{c}	1182^{c}	1173^{b}	1952^{cd}	−232^{b}	274^{d}	10	1667^{c}	1194
AAS	448^{e}	1687^{a}	1793^{a}	1648^{a}	1907de	168^{d}	546^{a}	–	1362^{f}	1089
DVE	921^{a}	1123^{e}	1240^{b}	1100^{c}	1664^{f}	184^{d}	113^{e}	9	1542^{d}	987
s.e.d.	37	39	47	36	65	68	10	–	61	–

[1] For abreviations see Table 2;
[2] Figures with different superscripts in a column differ significantly ($p < 0.05$);
[3] Microbial PU supply based on rumen available energy, rumen degraded CP, and the actual microbial PU supply (average smallest microbial PU);
[4] Rumen protein balance.

pared to other systems. The predicted milk protein production varied from 945 g d^{-1} in the DCP-system to 1194 g d^{-1} in the MP-system.

Predictability of milk protein production

The relationship between milk protein production and total PU intake was highest for the AAT-system (R^2 = 0.82) and decreased in the following order: AAS (0.81), CP (0.79), AP (0.78), PDI (0.75), DCP and MP (0.71) and DVE (0.61). Total NEL

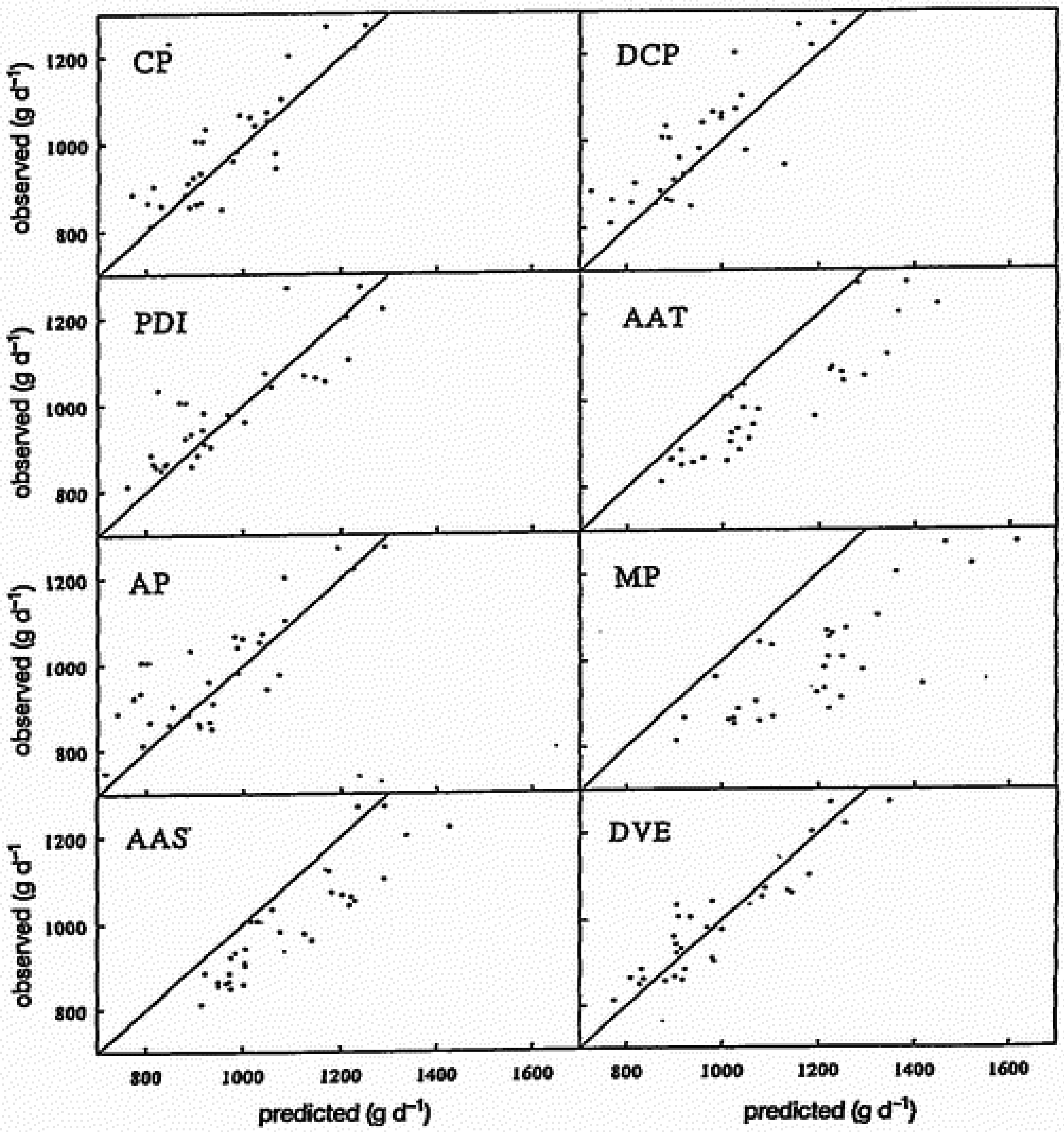

Figure 1. Relationships between observed and predicted milk protein production in the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system (line indicates Y = X).

intake showed a poor association with milk protein production ($R^2 = 0.49$). The relationship with milk protein production did not increase when PU and NEL intake were used in multiple regression.

The relationships between observed and predicted milk protein production are vizualised in Figure 1. Table 5 gives the APE, RPE and the decomposition of the MSPE. The average APE in the CP, DCP, PDI, AP and DVE-system was lower than in the AAT and AAS-system. The MP-system showed the highest APE. The RPE was lowest for the DVE-system and increased in the order of the CP, PDI, DCP, AP, AAS, AAT and MP-system. In the CP, DCP, PDI, AP and DVE-system most error was unexplained. Second highest source of error in the CP and DCP-system was due the difference of the intercept from 0, while in the PDI and DVE-system this was due to deviation of the regression slope from 1. In the AAT, MP and AAS-system most error was due to difference of the intercept from 0.

The most important correlation coefficients between the APE and feed intake variables are given in Table 6. The APE in the PDI, AAT and AAS-system were negatively related to the RPB. For all modern systems, APE was positively related to the escape PU intake and in the AAT and AAS system also to the microbial PU intake and NEL intake. The NEL-balance showed a relationship with the APE of all modern systems, except for the DVE-system. In all systems the APE had a close correlation between the PU available for milk production per NEL available for milk production.

Discussion

The escape protein fraction of feedstuffs was not measured in the experiments but were taken from tables that were published with the systems. This approach was preferred above using one escape fraction for each feedstuff whatever the system, be-

Table 5. Absolute and relative prediction error of milk protein production, and contributions of bias, regression and disturbances to the relative prediction error in the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system.

System[1]	Prediction error Absolute (g/d)	Relative (%)	Relative error due to Bias (%)	Regression (%)	Disturbance (%)
CP	−22	6.7	11.1	2.3	86.6
DCP	−44	8.8	25.6	4.4	70.0
PDI	−19	7.8	6.1	24.9	69.0
AAT	112	13.4	70.4	13.2	16.4
AP	−37	9.3	16.3	15.4	68.3
MP	204	22.9	81.0	8.5	10.5
AAS	100	11.7	74.8	4.1	21.1
DVE	−2	5.7	0.2	23.7	76.1

[1] For abbreviations see Table 2.

Table 6. Correlation coefficients between the absolute prediction error of milk protein production (APE) and the intake of rumen CP balance (g d^{-1}), escape PU (g d^{-1}), microbial PU (g d^{-1}), NEL (Mcal d^{-1}), NEL balance (Mcal d^{-1}) and PU/NEL available for milk production (g $Mcal^{-1}$) for the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system (n = 30).

	Intake					
System[a]	Rumen CP Balance	Escape PU	Micro-bial-PU	NEL	NEL-balance	PU/NEL
CP	–	–	–	-0.11^{ns}	0.18^{ns}	0.82^{**}
DCP	–	–	–	-0.28^{ns}	-0.04^{ns}	0.86^{**}
PDI	-0.64^{**}	0.61^{**}	0.13^{ns}	0.42^{*}	0.51^{**}	0.84^{**}
AAT	-0.65^{**}	0.64^{**}	0.53^{**}	0.70^{**}	0.53^{**}	0.70^{**}
AP	0.15^{ns}	0.77^{**}	0.08^{ns}	0.11^{ns}	0.37^{*}	0.90^{**}
MP	0.32^{ns}	0.60^{**}	0.12^{ns}	-0.24^{ns}	-0.57^{**}	0.76^{**}
AAS	-0.52^{**}	0.40^{**}	0.37^{*}	0.54^{**}	0.74^{**}	0.69^{**}
DVE	-0.33^{ns}	0.65^{**}	0.03^{ns}	0.17^{ns}	0.14^{ns}	0.79^{**}

[a] For abbreviations see Table 2;
^{ns} = not significant; * = $p < 0.05$; ** = $p < 0.01$.

cause in a given system coefficients are based on the corresponding escape protein fraction (Waldo and Glenn, 1984). Only for some less important concentrate ingredients, escape fractions were lacking in some systems and were subsequently taken from CVB (1991).

The high actual microbial PU in the AAS-system, was the result of a high microbial PU supply from both rumen available energy and rumen available CP. The high microbial PU supply from rumen available CP was caused by the low escape fractions and high recycling capacity (20% of microbial CP synthesis) in this system. However, total AAS supply was comparable to the PU supply in other systems, because this high microbial PU supply was compensated by low escape PU supply. Because of high maintenance requirements, a relatively small amount of the AAS supply was available for milk production, but this was compensated by the high efficiency of milk protein production, resulting in a predicted milk protein production comparable to other systems. Actual microbial PU supply in the AP-system was in all treatments limited by rumen degraded CP, and comparable to other systems. The AP-system therefore highly overestimates the microbial PU supply based on TDN in the diet, which was reflected in the highly negative RPB. To a lesser extend this also was true for the MP-system.

In the AAS and DVE-system, total PU supply was calculated from escape and microbial PU decreased with endogenous duodenal AAS (on average 188 g d^{-1}) or DVE required to compensate for metabolic faecal losses (on average 357 g d^{-1}). Adding the latter to the total PU intake resulted in values comparable to those in the other systems (2095 g AAS d^{-1} and 2021 g DVE d^{-1}). The average maintenance requirements were higher in systems where it was mainly based on feed intake (AP, AAS and DVE-system) than in systems where it was related to animal weight (PDI, AAT and MP-system). The low requirements in the MP-system compared to the other systems was caused by the high efficiency of MP use for maintenance purposes (1.00) compared to the other systems with a factorial approach of the maintenance requirements (0.67 in the AP and DVE-system).

High correlation coefficients between PU intake and milk protein production for the CP and DCP-system, comparable to the AAT-system, were also observed by Syrjälä-Qvist et al. (1985). However, Theun and Vik-Mo (1985) and Vik-Mo (1985) obtained better relationships with the AAT and PDI-system than with the DCP or CP-system. Variable results were obtained with the AP-system: Robinson et al. (1991) concluded that the NRC (1985) recommendations were too high, but Broderick et al. (1991) observed an increased milk protein production after increased AP intake. The AFRC (1992) reported, if variation between experiments was accounted for, a close association ($R^2 = 0.94$) between milk protein production and MP intake, which is in contrast to the poor results obtained with our dataset. According to AFRC (1992), MP was closely related to the PU calculated according to ARC (1984). Recommendations of the ARC (1984) were supported by Cody et al. (1991) and Robinson et al. (1991), but found to be too low by MacRae et al. (1988) and Vik-Mo (1985). The difference in performance within a system as reported in the literature is hard to explain. Variable results might be caused by differences in feedstuffs and protein escape fractions used, energy and PU supply relative to requirements and milk production level.

In contrast with the MP-system, the correction of DVE supply for retention and mobilisation of body protein resulted in a better prediction of milk protein production. The DVE-system showed, in contrast to the other modern systems, no significant relationship between the APE and NEL-balance. For those systems including protein retention or mobilisation in the system might increase the predictability of milk protein production.

The higher APE in the AAT and AAS-system compared to the PDI and DVE-system could be explained by the higher efficiency of milk protein synthesis applied in the AAT and AAS-system. In the MP-system the high APE compared to the other systems was a combined effect of high MP intake, low MP maintenance requirement and high efficiency of milk protein production. In all modern systems the APE showed a close relationship with escape feed PU, from which can be concluded that variation in escape PU supply has less influence on milk protein production than expected. This can be due to overestimation of the escape fraction compared to *in vivo* data. Another explanation would be that the amino acid profile of the escape protein was not in balance with the profile of amino acids needed for milk protein production.

In all systems a close positive relationship was observed between the APE and the ratio of PU/NEL available for milk production. The APE increased with increasing PU/NEL, which suggests that at higher PU intakes the efficiency at which PU is used for milk protein production decreases and illustrates the importance of the interaction between protein and energy at metabolic level (MacRae et al., 1988). Part of this decrease could be explained by the fact that in the NEL-system the NEL requirement per kg of fat and protein corrected milk (FPCM) increases with milk production:

NEL for milk in MJ = 3.04*FPCM + 0.005*FPCM^2.

In contrast to the NEL-system, protein evaluation systems assume a constant efficiency. The observed efficiency could be calculated by dividing milk protein production by PU available for milk production. As with the APE, the observed efficiency showed a close relationship with PU/NEL available for milk production. Additional variation in observed efficiency could be explained by including fat protein corrected milk production (FPCM) in the equation. For the DVE-system the following equation was developed ($R^2 = 0.75$, n = 30):

$$\underset{(g\,g^{-1})}{\text{efficiency}} = 1.22 - 0.031 * \underset{(g\,MJ^{-1})}{\text{DVE/NEL}} - 0.0032 * \underset{(kg\,d^{-1})}{\text{FPCM}} \qquad (1)$$

The decrease in efficiency agrees with the commonly accepted diminishing returns with increasing protein intake (MacRae et al., 1988). With equation (1) the efficiency was predicted for the treatments in the original dataset that were not used for the validation. Despite the fact that all those treatments were overfed in DVE, the predicted efficiency (range 0.43 to 0.69) showed a close relationship with the observed efficiency (range 0.42 to 0.63) ($R^2 = 0.84$, n = 40).

Milk protein production can be calculated from the DVE supply for milk production multiplied with the efficiency. Using equation (1), milk protein production can be calculated according to:

$$\text{milk protein} = (1.22 - 0.031 * \text{DVE/NEL} - 0.0032 * \text{FPCM}) * \text{DVE} \qquad (2)$$

The DVE/NEL ratio for milk production, at which maximum milk protein is achieved, can be calculated by putting the first derivative of equation (2) equal to 0, which results in:

$$\frac{\text{DVE}}{\text{NEL}} = \frac{1.22 - 0.0032 * \text{FPCM}}{0.031 * 2} \qquad (3)$$

With equation (3) it can be shown that DVE to NEL ratio at maximum milk protein production decreases from 19.2 at 10 kg FPCM d^{-1} to 17.6 at 40 kg FPCM d^{-1}, from which can be concluded that the increase in requirements with increasing FPCM production level is less pronounced for DVE than for NEL.

Conclusions

From the previous discussion it can be concluded that the prediction of milk protein production under Dutch conditions decreased in the order: DVE, CP, PDI, DCP, AP, AAS, AAT and MP-system. Predictions can be improved when a variable efficiency of milk protein production is used. In the DVE-system the observed efficiency decreased with increased protein to energy ratio in the diet and milk production level.

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