# Calculation of matrix values for a blend of essential oils in dairy cow rations

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Responsible and more efficient utilisation of feedstuffs in milk production is one of the main challenges in terms of sustainability and environmental impact.

The effects of a commercial blend of essential oils (BEO, Crina Ruminants) on various variables of ruminal fermentation, microbial community, and digestibility of individual nutrients have been evaluated in numerous in vivo and in vitro studies.

Effects on milk yield and milk composition have been determined. It has been shown that supplementation of BEO increased daily milk production and production of milk protein by optimising ruminal metabolism via selective influences on microbial composition in the rumen.

The objectives of this study were therefore to define the term matrix value (MV), which has not been in use in ruminant nutrition to date, and to calculate MVs that describe the benefits of BEO on milk and milk protein production.

These MVs served to evaluate the potential for saving feed energy and intestinally-available protein through inclusion of BEO in the rations of dairy cows.

#### **Materials**

Animal Care and Use Committee approval was not obtained for this study because data were obtained from existing studies by Varga et al. (2004; control n = 257, BEO-receiving n = 248) and Offer et al. (2005; n = 16 Holstein-Friesian cows in early lactation). The BEO tested in those studies also contained thymol, eugenol, vanillin, and limonene on an organic carrier.

Optimisation of ruminal metabo-

lism by means of BEO is achieved by inhibition of hyper-ammonia producing bacteria and probably also via toxicity of BEO to Ruminobacter amylophilus, although BEO has no effect on the colonisation of starchrich substrates by this species.

As a result, rates of ruminal amino acid and presumably also of starch degradation are decreased and may account for better synchrony between energy and protein degradation. Furthermore, ruminal concentration of ammonia is decreased and decline of pH after feeding is reduced.

#### **Defining matrix values**

First of all, the term matrix value (MV) needs to be defined. A suitable definition is used in pig and poultry nutrition for the enzyme phytase whereby the 'phosphorus equivalence value' of this enzyme is based on the assumption that a certain amount of phosphorus may be spared because of the inclusion of phytase in the diet.

The MV of phytase is therefore equal to the amount of inorganic phosphate that is generated or liberated, respectively, when a certain amount of phytase is added.

Use of the above definition with regard to BEO is critical because BEO does not liberate a specific

Study	Matrix values	Dose of BEO', g/animal /day	Results animal/day
Offer et al., 2005²	Milk production/ NE saving	0.5 1.0 2.0	+ 1.4kg milk <sup>3</sup> = 1.1 kg FCM <sup>4</sup> + 1.7kg milk <sup>3</sup> = 1.5 kg FCM + 2.0kg milk <sup>3</sup> = 1.6 kg FCM
Offer et al., 2005	Milk protein production/ Intestinally- available protein saving	0.5 1.0 2.0	+ 57g milk protein + 69g milk protein + 76g milk protein
Varga et al., 2004	Milk production/ NE saving	1.2	+ 1.5 kg FCM (P<0.05)

IBEO = blend of essential oils (Crina Ruminants). <sup>2</sup>In the study of Offer et al. (2005) cows received 12kg fresh weight of a protein concentrate/animal/day, whereas grass slage was fed ad libitum. The increase in milk and milk protein production was linear (P = 0.031 and 0.015, respectively). <sup>3</sup>Values for absolute milk production were transferred to FCM. <sup>4</sup>FCM = fat-corrected milk (4%).

Table 2. Results of studies selected for the calculation of matrix values.

substance, but instead affects among other things ruminal protein as well as carbohydrate metabolism.

Nevertheless, an MV for BEO can be defined as the amount of a specific nutrient that is additionally available to the ruminant when BEO is fed.

The increase of the available amount of a particular nutrient may be used to increase the animal's performance.

Alternatively BEO may be fed to save nutrients whilst production is

Table I. Matrix values of special interest in dairy cattle for a blend of	
essential oils (Crina Ruminants).	

ltem	Matrix value
Milk production	Amount of additional milk that is produced when BEO is included in the diet: +Milk, +FCM 4%
Milk protein	Amount of additional milk protein that is produced when BEO is included in the diet: protein
NE	Amount of additional feed energy that is available or may be saved by inclusion of BEO in the diet whilst maintaining previous level of production
Intestinally-available protein: PDI <sup>1</sup> , nRP <sup>2</sup> , MP <sub>G8</sub> <sup>3</sup> , DVE <sup>4</sup> , MP <sub>USA</sub> <sup>5</sup>	Amount of additional intestinally-available protein that is available or may be saved when BEO is included in the diet whilst maintaining previous level of production
<sup>1</sup> PDI = intestinally-digestible protein <sup>4</sup> DVE = true protein digested in the	$\label{eq:constraint} \begin{array}{l} (\mbox{French system}). \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$

maintained at the pre-BEO level. Both, the saving of nutrients as well as the increase in production, may represent MVs for specific dosages of BEO.

#### **Variables for calculation**

Because BEO has a variety of effects on variables of (ruminal) metabolism as well as on the animal's performance, it is conceivable that MVs could be defined for an entire set of variables. For example, in dairy cows it is possible to define MVs for individual nutrients based on their digestibility, for individual milk components, or for energy content of milk.

Nevertheless, not all MVs that could theoretically be computed are of use in practice. An MV for milk production provides valuable information by predicting the amount of additionally produced milk on account of BEO, whereas an MV that gives the amount of acid detergent fibre (ADF) that may be saved because of its improved digestibility with BEO is of little practical value to a producer.

In dairy nutrition, MVs for milk and milk protein production but also for NE and intestinally-available protein *Continued on page 14*  Continued from page 13 are of particular interest. Definable MVs are shown in Table 1.

#### **Selection of studies**

From a practical standpoint, it would be an advantage to have MVs available that describe the increased value of a diet in terms of net or metabolisable energy and intestinally available protein according to the respective feed evaluation systems of the particular countries. Variables such as lower deaminase activity and improved productions of total volatile fatty acids (VFA) as well as the individual VFA acetate and propionate illustrate the mode of action of BEO in the rumen.

However, there is no practical way of incorporating theses variables into any equations in the feed evaluation systems in a meaningful fashion, despite the fact that they are influenced significantly (P<0.05) by additions of BEO.

In contrast, it appears feasible to make practical use of results of digestibility trials and in vivo trials that evaluate the effects on variables such as lactation performance or body weight gain.

Nevertheless, the observed effects of BEO are not always significant and in some cases the results of individual studies are conflicting. For that reason, non-significant ( $P \ge 0.05$ ) and conflicting results were excluded from the calculation of MVs in the present study.

In conclusion, results most suitable for the calculation of MVs were published by Varga et al. (2004) and Offer et al. (2005) who were able to document increased productions of milk and milk protein as a result of inclusion of BEO in the ration.

#### Feed evaluation systems

Actually, the calculations of energetic and protein value of ruminant feeds differ in the individual systems of France, Germany, Great Britain, the Netherlands, and the USA.

#### Energy evaluation systems

In the four mentioned European systems calculation of metabolisable energy (ME) in a feed is based on GE content and, in France, also on the content of organic matter (orgM).

The obtained ME value of the feed is corrected for crude protein (CP) content in Germany, and for sugar in the Netherlands, respectively, whereas the French and the British systems correct the ME value for the level of intake.

The factor 'k', which describes the efficiency of utilisation of ME, is used to convert ME into the net energy (NE) lactation values in the French, German, and Dutch systems, but not in the British system; the latter



Fig. 1. Increases in milk production with the inclusion of a blend of essential oils (BEO, Crina Ruminants) in the diet of dairy cows in the studies of Offer et al. (2005) and Varga et al. (2004). 'FCM 4% = fat-corrected milk 4%.

terminates the calculation of the energy content on the level of ME. After that the Dutch system corrects the NE value for the level of intake.

In contrast to the German, the British, and the American systems the Dutch and the French systems also provide for the use of feed units [feed unit lactation (VEM) in the Dutch system and feed unit for dairy cattle (UFL) in the French system] instead of NE values.

In contrast, the American Cornell system uses the content of total digestible nutrients (TDN) to evaluate the amount of digestible energy (DE), which is then corrected for the level of intake.The DE then serves as the basis for calculation of ME and NE content on the animal's individual production level.

In conclusion, comparison of these five systems reveals differences in the calculations themselves as well as in the methodology of the systems. For example, the German and the Dutch systems, use a fraction called N-free extract substances for calculation of the energy content, whereas the French system prefers the use of ADF and neutral detergent fibre (NDF) to characterise the carbohydrate fraction.

Accordingly, the study by Vermorel and Coulon (1998) revealed differences in ME and NE values in the German, British, and Dutch systems compared with the French system ranging from -0.5  $\pm$  1.9 to 0.3  $\pm$  3.5%.

Concentrates tend to have higher NE values in the Dutch and the French systems compared with the German system.

Values for the American system can not be deduced from the study by Vermorel and Coulon (1998) as this system was completely renewed by NRC in 2001.

#### Protein evaluation systems

The protein evaluation systems of France, Germany, Great Britain, the Netherlands, and the USA all consider the amount of protein leaving the rumen undigested by microbes [PDI of nutritive origin (PDIA) in France; non-ruminal degraded protein (UDP) in Germany; UDP and digestible undegraded protein (DUP) in Great Britain; undegraded feed CP (BRE) in the Netherlands; RUP in the USA] and the effect of energy on synthesis of microbial protein [contents of fermentable organic matter (forgM) in France; ME or digestible organic matter, (dorgM) in Germany; fermentable ME (FME) and ADIN in Great Britain; dorgM in the Netherlands; TDN in the USA].

Thus, with the exception of the German system all systems deter-

Fig. 2. Increases in milk protein production with inclusion of a blend of essential oils (BEO, Crina Ruminants) in the diet of dairy cows in the study of Offer et al. (2005).



mine a precise value for the amount of microbial protein synthesised in the rumen [PDI of microbial origin (PDIM) in France; digestible microbial true protein (DMTP) in Great Britain; rumen-synthesised microbial protein digested in the small intestine (DVME) in the Netherlands; microbial CP (MCP) in the USA].

After that, the French system adjusts the calculated value with theoretically synthesisable microbial protein dependent on nitrogen and energy content in the diet.

Nonetheless, both the Dutch and the American systems consider endogenous nitrogen losses as functions of the digestible fraction of crude ash (VRAS) and dorgM or dry matter intake (DMI), respectively.

Apart from the individual methodical approaches of the systems, total CP is in some cases divided into further fractions before calculation and not all fractions are necessarily determined by the same analytical methods.

To our knowledge, there are no studies comparing the current feed energy and feed protein evaluation systems.

The established methodical differences as well as the outcomes of the study by Vermorel and Coulon (1998) indicate that calculation of MVs for NE and intestinally-available protein must be determined individually for each system. For this reason MVs from different systems are not interchangeable without accepting an error of unknown extent.

#### **Methods**

Results chosen for the calculation of MVs are shown in Table 2. Increases in milk and milk protein production were expressed as functions of the dosages of BEO included in the dairy cow rations.

Appropriate equations to link the data points were determined by means of the spreadsheet MS Office Excel 2003.

#### **Milk production**

Fig. I shows the correlation between the daily dosages of BEO and the increase in milk production (+FCM 4%) in the studies of Varga et al. (2004) and Offer et al. (2005). Although the polynomic function +FCM 4% =  $0.8452x^3 - 3.425x^2 +$ 4.4583x - 0.3786 links all data points in Fig. I optimally, it seems unlikely that milk production would follow this curve.

The same applies to the quadratic function +FCM  $4\% = -0.3657x^2 +$ 1.239x + 0.5806. The logarithmic (+FCM  $4\% = 0.3654 \ln (x) +$ 1.4083) or the even more simple linear (+FCM 4% = 0.3019x + 1.0702) function are most likely to reflect the

effect of BEO on milk production

and it is possible to determine the

Matrix value NE, MJ NE/animal/day						
	Calculation based on matrix values		Calculatio	Calculation based on matrix value		
System	Eq i'	Eq i"	Eq i"	Eq ii'	Eq ii"	Eq ii"
French	3.82 = 0.54 UFL <sup>1</sup>	4.29 = 0.60 UFL'	5.23 = 0.74 UFL'	3.63 = 0.51 UFL <sup>1</sup>	4.41 = 0.62 UFL'	5.20 = 0.73 UFL
German	4.03	4.52	5.51	3.83	4.65	5.48
British	6.36 <sup>2</sup>	7.15 <sup>2</sup>	8.71 <sup>2</sup>	6.05 <sup>2</sup>	7.36 <sup>2</sup>	<b>8.66</b> <sup>2</sup>
Dutch	3.73 = 541 VEM <sup>3</sup>	4.18 = 606 VEM <sup>3</sup>	5.10 = 791 VEM <sup>3</sup>	3.54 = 513 VEM <sup>3</sup>	4.31 = 625 VEM <sup>3</sup>	5.07 = 738 VEM <sup>3</sup>
USA	3.82	4.29	5.23	3.63	4.41	5.20

<sup>1</sup>UFL = feed unit for dairy cattle (French system). <sup>2</sup>Values for the British system are given as MJ ME/animal/day, k (efficiency of utilisation of ME for lactation) for calculation of ME was set at 0.6. <sup>3</sup>VEM = feed unit lactation (Dutch system).

Table 3. Matrix values that describe the amount of feed energy that may be saved with inclusion of a blend of essential oils (Crina Ruminants) in the diet whilst maintaining previous levels of production (matrix value NE) in individual feed evaluation systems.

increases in milk production for each dose of BEO between 0.5 and 2.0g/animal/day using the linear (i) as well as the logarithmic (ii) function:

+FCM 4% = 0.3019/BEO +1.0702	(i)
+FCM 4% = 0.3654/In (BEO) + 1.4083	(ii)

Analogous to the phosphorus equivalence value this allows the use of several fixed or static MVs in practice. Because milk production does not increase to the same extent as the dosages of BEO in these two studies, it seems reasonable to define static values (+FCM  $4\%_{xgBEO}$ ) for relatively low dosages by inserting specific additions of BEO (g/animal/day) into Eq. i and ii:

+FCM 4‰.5g BEO = 1.22 kg FCM 4%/animal/day (i')
+FCM 4%.og BEO = 1.37 kg FCM 4%/animal/day (i'')
+FCM 4‰ <sub>20g BEO</sub> = 1.67 kg FCM 4%/animal/day (i''')
+FCM 4‰.5g BEO = 1.16 kg FCM 4%/animal/day (ii')
+FCM 4%g BEO = 1.41 kg FCM 4%/animal/day (ii'')
+FCM 4%2.0g BEO = 1.66 kg FCM 4%/animal/day (iii''')

### Net energy for lactation

If MVs that describe the increase in milk production with BEO can be defined, it is also possible to calculate the amount of energy in the ration that is saved with the inclusion of BEO, assuming that milk production is maintained at the pre-BEO level. In the individual feed evaluation systems, the amount of energy needed for production of 1 kg of FCM 4% differs and ranges from 3.054 MJ NE in the Dutch system, 3.13 MJ NE in France and the USA to 3.30 MJ NE in the German system. In the British system, it is assumed that 1 kg of FCM 4% contains 3.13 MJ NE. This value needs to be divided by k, which is the efficiency of utilisation of ME for lactation, to obtain the corresponding British ME value.

The combination of MVs for milk production (i', i'', i''', ii', ii'') and the energy requirement for its production allows the determination of the MVs for feed energy savings shown in Table 3.

#### Milk protein yield

Analogous to milk production, the results of the study by Offer et al. (2005) can be used to calculate MVs for milk protein yield (Table 2).

Fig. 2 illustrates possible mathematical correlations between the individual values obtained with three different dosages of BEO.

It is unlikely that the negative quadratic function +Milk protein =  $-11.333x^2 + 41.000x + 39.333$ describes the effect of BEO on milk protein production correctly. For further calculations, it is therefore reasonable to use the linear as well as the logarithmic function (Eq. iii and iv):

+Milk protein = 11.857x + 53.500	(iii)
+Milk protein = 13.706 ln (x) + 67.333	(iv)

Equations iii and iv provide the opportunity to define MVs for the increase in milk protein production for individual doses of BEO, analogous to milk production. Calculated values can be taken from Eq. iii' till iv'':

+Milk protein0.5gBEO = 59.4g	milk
protein/animal/day	(iii')
+Milk proteingBEO = 65.4g	milk
protein/animal/day	(iii'')
+Milk protein20gBEO = 77.2g	milk
protein/animal/day	(iii''')
+Milk protein05gBEO = 57.8g	milk
protein/animal/day	(iv')
+Milk protein.0gBEO = 67.3g	milk
protein/animal/day	(iv")
+Milk protein20gBEO = 76.8g	milk
protein/animal/day	(iv''')

#### Protein

The protein requirement for additional production of milk protein can be calculated by means of equations used to evaluate the protein requirements in the individual evaluation systems of France, Germany, Great Britain, the Netherlands, and the USA.

Table 4 lists the corresponding equations and shows theoretical values for protein requirements calculated for additionally produced milk protein in the particular systems based on MVs +Milk protein in Eq. ii' to iv'''.

These values also represent the amount of intestinally-available protein that may be saved due to addition of BEO.

#### **Summary of results**

From the inclusion of BEO at dosages of 0.5 to 2.0g BEO/ animal/day in dairy rations, a theoretical increase in milk production by 1.16 to 1.67kg FCM 4%/animal/day can be calculated.

The energy needed for the production of these amounts of milk reflect the MVs NE, which range from 3.63 to 5.23, 3.83 to 5.51, 3.54 to 5.10, and 3.63 to 5.23 MJ NE/ animal/day in the French, German, Dutch, and American systems, respectively.

In Great Britain, the corresponding MVs range from 6.05 to 8.71 MJ ME (Table 3). Furthermore, it may also be possible to increase milk protein production by 57.8 to 77.2g/ animal/day by adding BEO at a dose of 0.5 to 2.0g/animal/day.

Matrix values that describe the possible savings of intestinally-available protein (PDI, nRP, MP<sub>GB</sub>, DVE, and MP<sub>USA</sub> - see Table 4) vary greatly in their absolute values compared with MVs NE depending on the evaluation system used.

The calculated values range from 74.7 to 107g PDI in the French system, and from 121-162g nRP/ani-mal/day in the German system.

#### **Economic benefit**

Although the addition of BEO at a daily dosage of 0.5g/animal/day has a positive effect on both production of milk and milk protein, the greatest effect appears to be achieved at a dosage of 1.0g/animal/day (Figs. 1 and 2).

Higher dosages of BEO may lead to further increases in milk and milk protein production or higher energy and intestinally-available protein savings, but doubling the dosage of BEO does not necessarily result in a doubling of additionally produced milk or milk protein.

Nevertheless, adding BEO to dairy cow rations at a level of 1.2g of BEO/animal/day may result in savings of \$0.27/animal/day (increased milk income minus feed cost, based on 2006 average producer prices for a Wisconsin dairy, calculation by M. D. Tassoul and R. D. Shaver, unpublished).

#### Challenges in the field

To date there have been no conclusive comparative data on the effects of BEO in cattle relative to breed, age, weight, lactation stage and number, and production level.

Tassoul and Shaver (2009) observed no benefit of BEO in prepartum cows, whereas in early lactation DMI was decreased but milk production was maintained at the level of the control group.

Therefore, it is considered possible, that conflicting results in some of the studies were attributable to different study designs.

Furthermore, the health status of the animals, and in particular, the composition of the diet may modify the effects of BEO in dairy cattle. It is therefore imperative to include a safety margin that accounts for individual effects of animals and diets *Continued on page 16* 

#### Matrix value intestinally-available protein, g PDI<sup>1</sup>, g nRP<sup>2</sup>, g MP<sub>GB</sub><sup>3</sup>, g DVE<sup>4</sup>, and g MP<sub>USA<sup>5</sup></sub> /animal/day Calculation based on Calculation based on Equation for calculation of matrix values matrix values protein requirement for milk protein production Eq iv" Eq iii" Eq iv" System Eq iii' Eq iii" Eq iv' $PDI = 1.56/PL^{6}/TP^{7}$ 74.7 107 74.7 90.4 French 90.4 107 125 137 German nRP = 2.1/Milk protein 162 121 141 161 British $MP_{GB} = True \text{ protein in milk}/0.68$ 87.4 96.1 114 85.0 99.0 113 DVE = 1.396/Milk protein production + Dutch 83.7 92.1 109 81.4 94.9 108 0.000195/(Milk protein production)<sup>2</sup> USA $MP_{USA} = (Milk protein/0.67)/1000$ 88.7 97.5 115 86.3 101 115

<sup>1</sup>PDI = intestinally-digestible protein (French system). <sup>2</sup>nRP = usable CP (German system). <sup>3</sup>MP<sub>GB</sub> = MP (British system).

<sup>4</sup>DVE = true protein digested in the small intestine (Dutch system). <sup>3</sup>MP<sub>656</sub> = MP (American system). <sup>4</sup>PL = milk production (kg, French system). <sup>4</sup>TP = protein content in milk (g/kg milk, French system).

## Table 4. Matrix values that describe the amount of intestinally-available protein (PDI, nRP, MP<sub>GB</sub>, DVE, and MP<sub>USA</sub>) that may be saved with inclusion of a blend of essential oils (Crina Ruminants) in the diet whilst maintaining previous levels of production in individual feed evaluation systems.

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when planning BEO-supplemented rations. For these reasons in particular, the MVs calculated in this study also need to be tested in the field. It is of particular interest, whether there are concurrent savings in energy and intestinally-available protein and whether these two factors are independent of each other. Initial field experience with the MVs of the individual feed evaluation systems confirmed a possible saving of 0.5 MJ NEi/animal/day.

Nevertheless, it should be noted that inclusion of BEO in the diet does not necessarily result in increased milk production or obvious energy savings because energy may also be used for body weight gain (especially in heifers), metabolic activity, foetal growth or reproduction. In addition, conversion of ME to NE differs as a function of feed quality and purpose of utilisation.

Unpublished results of an in vitro study that tested the MVs for nRP calculated in the present study using the Hohenheim gas test revealed that 8g nRP/kg DMI may be spared by adding of BEO to a diet.

With an assumed daily DMI of 12kg/animal, the corresponding

amount of 96g nRP includes a safety margin of about 30% compared with the calculated value (Table 4, dosage of 1g BEO/animal/day) and is equivalent to the protein content in 1.25kg FCM 4%.

However, determination of this value was achieved in an in vitro system and only considered concentrated feeds.

#### Conclusion

The findings of this study suggest that there are linear and logarithmic correlations between the amounts of BEO included in the ration and the amounts of additionally produced milk and milk protein.

These gains could be converted to savings in feed energy and intestinally-available protein. Increases in production, as well as potential feed savings, could be expressed in the form of MVs.

However, MVs calculated in the present study require testing in the field to determine whether savings of energy and intestinally-available protein occur simultaneously.

Furthermore, it is necessary to control for the effects of stage of lactation and metabolic state of the animal.

References are available from the authors on request