Estimates for rumen dry matter degradation of concentrates are higher, but not consistently, when evaluated based on *in sacco* as compared to *in vitro* methods

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Introduction

NorFor – the Nordic feed evaluation system - currently bases estimates of ruminal crude protein and NDF degradation on data from the *in sacco* nylon bag technique. These degradation profiles are fitted to an exponential model with an intercept as proposed by Ørskov and McDonald (1979), where the intercept represents the soluble part of the substrate, while the asymptote is the potentially degradable part (Åkerlind *et al.,* 2011). The *in vitro* gas production technique (IVGPT) is a method used to estimate fermentation kinetics of feeds, by incubating the feeds in buffered rumen fluid and measuring the gas produced over time (Theodorou *et al*., 1994). Cumulative gas production profiles have been related to dry matter (DM) degradation, by assuming a potentially degradable pool equal to the amount of substrate degraded at the end-point of the fermentation and a constant yield factor of gas to degraded substrate (Dhanoa *et al.,* 2000). The aim of this study was to examine whether DM degradation estimated from mathematical models fitted to degradation profiles of concentrates obtained through the *in sacco* technique correspond to estimates by the rapid and less costly IVGPT.

Materials and Methods

IVGPT

Nine concentrates were tested in two *in vitro* gas production trials. The concentrates were ground, using a cyclone mill (Cyclotec 1093 sample mill, Foss Analytical A/S, Hillerød, Denmark), to pass a 2-mm screen and run in quadruplicates in each trial. Samples of 500 ± 1 mg were weighed into 100-ml glass jars. Rumen fluid with particulate matter was collected from two rumen cannulated, overnight fasted Jersey heifers on the morning of the experiment and transported for approximately 45 minutes in four pre-heated thermo bottles. The rumen fluid was then filtered through 2 layers of cheesecloth and added to a pre-heated (39°C) buffered solution (1:2 ratio) (Mencke & Steingass, 1988) that was kept anaerobic by continual CO_2 flushing. Ninety ml of the buffered rumen fluid solution was dosed into each of the 100 ml jars, and three jars without substrate were included for blank correction. The jars were placed in a preheated (39°C) thermoshaker with 40 rotations pr. minute. The ANKOM^{RF} Gas Production System (Ankom Technology, Macedon NY, US) was used to register gas production every 10 minutes for each sample during the fermentation period, with gas released at a pressure of 0.0517 bars. After 9 hours of incubation, half of the samples were removed, and the medium filtered through pre-weighed 25 µm F57 filter bags (ANKOM Technology). The remaining samples were removed and filtered through F57 bags after 48 hours of fermentation. Samples removed after 9 hours had an average of 54 gas readings, while samples removed after 48 hours had an average of 288 gas readings. One sample of palm expeller incubated for 48 hours was excluded due to the filter bag breaking during filtering. After drying, the undegraded residue was determined after correcting for microbial weight gain in the blank bottles.

In sacco nylon bag technique

The same nine concentrates were ground to pass a 1.5-mm screen using a cutter mill with four stationary and three rotating knives (Model 880803, Brabender OHG, Duisburg, Germany). Samples of 1700 ± 5 mg were weighed into 35-µm polyester bags (Saatifil PES 28/31, Saatitech S.p.A., 22070 Veniano, Italy). The bags were soaked in 39°C tap water for 20 minutes prior to incubation. The samples were incubated in the rumen of three non-lactating dairy cows fed at maintenance level, for 0, 2, 4, 8, 16, 24 and 48 hours, respectively. Two bags of each sample for each timepoint were incubated in each cow giving a total of six replicates for each feedstuff at each timepoint. After incubation, the bags were washed in a domestic washing machine, dried at 45° C and weighed. The bags were then emptied, vacuum cleaned and weighed to calculate the weight of air-dried sample residue.

C*omputational analysis*

Gas production profiles of the samples from the IVGPT were converted into ml gas $(g\ DM)^{-1}$ at Standard Pressure and Temperature (STP). A mean cumulative gas production profile for each feed was estimated from replicates incubated *in vitro* for 48 hours and mean dry matter degradation was calculated based on undegraded feed residue after 9 and 48 hours of incubation. The program R (R Core Team, 2019) with the "drc-package" (Ritz *et al.*, 2015) was used to fit eight mathematical models to the gas production and feed degradation profiles obtained *in vitro* and *in sacco*, respectively.

After fitting the observed curves, the in vitro results were converted from ml gas produced to percentage of DM degraded. This was done by assuming a potential DM degradation equal to what was degraded after 48 hours of incubation and a constant yield factor (ml gas $(g\ DM)^{-1}$).
The estimated gas production at time 't' was then divided by the yield factor and reported as a proportion of the original sample DM.

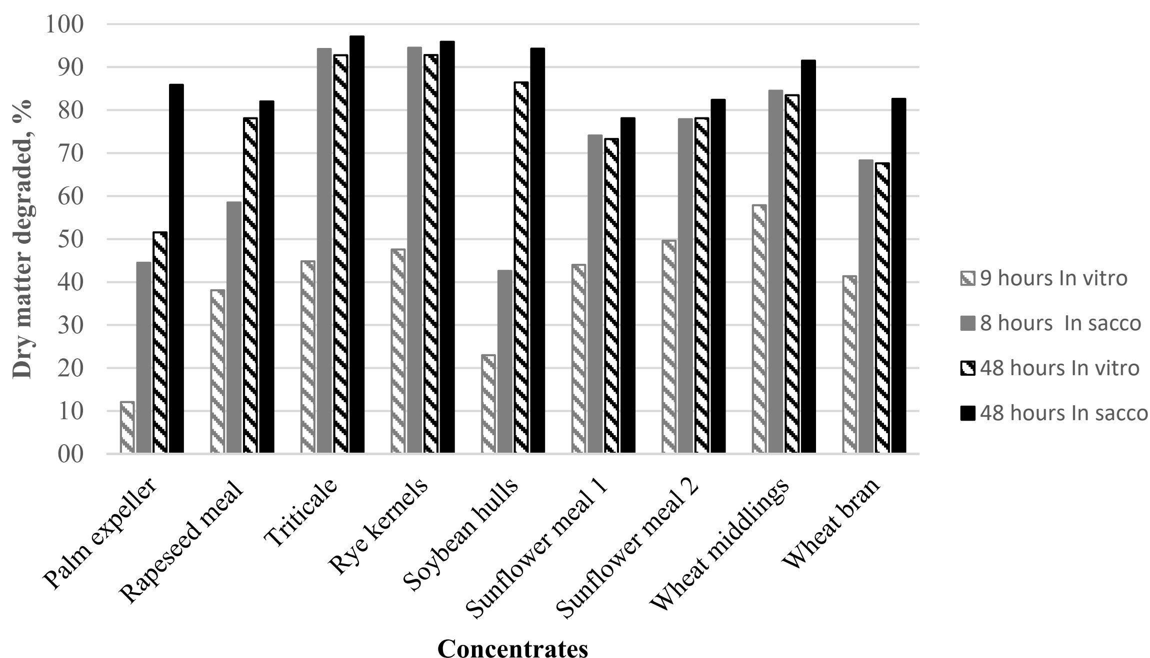
The eight models initially fitted to each degradation profile are in Table 1. The best fit was selected based on AIC (Akaike's Information Criterion) and used to calculate total rumen dry matter degraded for both the *in vitro* and the *in sacco* degradation profiles of each concentrate, assuming a mean retention time in the rumen of 16 hours.

This estimated rumen retention timed was derived from the equation used in NorFor to calculate passage rate of potential degradable starch and crude protein from concentrate (Åkerlind *et al.,* 2011), assuming a dairy cow with a body weight of 640 kg, a daily DM intake of 22 kg and a concentrate share of 50% of the feed ration.

Table 1 Mathematical models fitted to degradation profiles of nine concentrates

Abbreviation	Model type	Equation
EXP	An exponential model	$Y_t = B*(1-e^{-ct})$
EXP.L	An exponential model with a lag phase	$Y_t = B*(1-e^{-c(t-L)})$
Ø & M	An exponential model with an intercept (Ørskov & McDonald, 1979)	$Y_t = A+B*(1-e^{-ct})$
EXP.IL	An exponential model with an intercept and a lag phase	$Y_t = A+B*(1-e^{-c(t-L)})$
Groot	A sigmoidal model (Groot *et al.*, 1996)	$Y_t = A/(1+(b^c/t^c))$
Weibull	A continuous probability distribution	$Y_t = A+B*\exp^{-\exp(b*(\log(t)-\log(e)))}$
Gompertz	A sigmoid curve with slow growth at the start and end of the curve	$Y_t = A+B*\exp^{-\exp(b*(t-e))}$
M-M	A Michealis-Menten curve	$Y_t = A+B/(1+(e/t))$

Results and discussion
Across feeds, DM degradation was significantly higher (*P*<0.05) for the samples incubated *in sacco* at eight and 48 hours compared to the samples incubated *in vitro* for nine and 48 hours, respectively (Figure 1). Mean DM degradation across feeds after 8 hours of incubation *in sacco* was 71.0 ± 19.4%, while it was 39.8 ± 14.1% after nine hours of incubation *in vitro.* Mean dry matter degradation across feeds after 48 hours of incubation *in sacco* was 87.8 ± 7.0%, while it was 78.2 ± 13.1% after 48 hours of incubation *in vitro.*



percentage of dry matter degraded after 9 and 48 hours of incubation, with the lowest correlation being for wheat middlings (r=0.94, *P*<0.005). Feed degradation profiles were best fitted to different mathematical models, depending on whether feeds had been incubated *in sacco* (Weibull, Ø & M or M-M) or *in vitro* (Groot, Gompertz and Ø & M), and in no case was the best fit model for a feed identical for the two methods (Table 2).

Table 2 Best fit mathematical models used to describe feed degradation profiles based on AIC, depending on concentrate and technique

Concentrate	In vitro	In sacco
Palm expeller	Ø & M	Weibull
Rapeseed meal	Groot	Weibull
Triticale	Groot	Weibull
Rye kernels	Gompertz	Ø & M
Soy bean hulls	Ø & M	Weibull
Sunflower meal 1	Gompertz	Ø & M
Sunflower meal 2	Gompertz	Ø & M
Wheat middlings	Groot	Weibull
Wheat bran	Groot	M-M

For rye seeds, sunflower meal and wheat middlings, DM degradation was very different in the early compared to the final stage of *in vitro* fermentation, which was not the case when incubated *in sacco* (Figure 1).
Estimated dry matter degradation, assuming 16 hours retention in the rumen and using the predicted degradation curves, was significantly greater (*P<0.05*) for *in sacco* compared to *in vitro* estimates for all concentrates (Table 3). There was a non-significant (*P*=0.66) smaller

mean standard deviation for estimated DM degradations based on degradation profiles obtained *in sacco* (SD =1.38 unit) compared to *in vitro* (SD =1.64). Use of the best fit model resulted in only minimal differences in degradation estimates at 16 hours rumen retention when compared to the other tested models (data not shown).

Table 3 Estimated rumen dry matter degradation (%) derived from *in vitro* and *in sacco* degradation profiles for 9 concentrates, according to the best fit model and assuming a mean rumen retention time of 16 hours

Concentrate	In vitro	In sacco
Palm expeller	21.6[a]	76.8[d]
Rapeseed meal	57.0[b]	72.9[d]
Triticale	73.3[b]	95.6[d]
Rye kernels	72.8[c]	95.5[a]
Soy bean hulls	44.3[a]	67.8[d]
Sunflower meal 1	59.7[c]	77.6[a]
Sunflower meal 2	63.0[c]	81.7[a]
Wheat middlings	69.9[b]	87.4[d]
Wheat bran	52.5[b]	73.0[e]

a, b, c, d, e: = Estimated using the Ø & M, Groot, Gomperts, Weibull and M-M models, respectively.

The reasons for the observed discrepancies between the two techniques are not known. Estimates for early degradation by the *in sacco* method are affected by amount of substrate escaping (but not necessarily degraded) at time zero (intercept) from the bags placed in the rumen (Huntington & Givens, 1995). Such an escape is not possible from bottles used *in vitro*. However, any initial loss of material from *in sacco* bags should not affect estimates of degradation rates for remaining feed in the bag, since several of the applied mathematical models included an intercept. Particle size of feed samples is another issue to consider, since feed samples used in the *in sacco* trial were ground through a smaller sieve size (1.5 mm) than those used *in vitro* (2 mm). However, the cyclone mill used for *in vitro* samples has been shown to create a smaller mean particles size compared to other mill types (Hoffman *et al*., 2012). A linear relationship has been shown between bag pore size and estimates of undegraded particle loss (Michalet-Doreau & Ould-Bah, 1992). There was a difference in pore size between the filter bags used *in vitro* (25 µm) and *in sacco* (35 µm). It is possible to use filter bags with smaller pore sizes in the *in vitro* system compared to the *in sacco* method, since the *in vitro* samples are incubated directly in the fluid and bags are only used to retain undegraded feed material at the end of the incubation. *In sacco* degradation requires bags with pore sizes large enough to allow for passage of microorganisms. This raises the question whether differences in degradation between the two methods to a large extent could be explained by loss of undegraded particles in the *in sacco* method, and subsequent inflated increase in estimates for degraded particles. Another factor that may contribute to uncertainties related to the IVGPT, is the microbial concentration and microbial activity in the rumen fluid. The activity and concentration are known to be affected by time of rumen fluid sampling, relative to the time when the cow was fed (Mould *et al*., 2005). The rumen fluid used in the *in vitro* system was collected from heifers that have been fasted overnight to obtain standardized results from run to run. A greater gas production in the early stages of fermentation could perhaps be expected, if the cows were recently fed. Less gas is produced from degradation of protein than degradation of carbohydrates (Cone & van Gelder, 1999). This means that gas production relative to unit of DM degraded is not constant. DM degradation in the *in vitro* system is estimated from amount of gas produced and DM degraded at the end of incubation (48 hours). Since soluble protein will mostly be degraded in the early fermentation (Cone & van Gelder, 1999), the deviation between estimated DM

degraded to actual DM degraded is expected to be larger at that stage. In the *in vitro* system DM degraded, and gas produced had a correlation ≥0.94 for all samples, considering both 9 and 48 hours of incubation. As the estimated rumen retention time was 16 hours, the effect of protein is assumed to be minimal.

Conclusions

In sacco estimates of DM degradation (assuming a 16 hour rumen retention time for all feeds) were significantly greater across feeds compared to *in vitro* estimates. These differences cannot readily be explained by the characteristics of the type of concentrate. There were no major differences in estimates for DM degradation depending on the mathematical model applied, when assuming a mean rumen retention time of 16 hours. Further studies are needed to determine which method most accurately describes differences between feeds with respect to dry matter degradation in the rumen, and whether the rapid and cheap *in vitro* method could complement normal feed analyses to refine description of degradation kinetics.

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