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# Comparison of fractionation methods for nitrogen and starch in maize and grass silages

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1. Introduction
2. Aim
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*In situ* method : used to estimate rumen degradation of nutrients in feed ingredients

Method based on substrate disappearance from nylon bags during incubation in the rumen and modelling according to a first order reaction

Degradation parameters are used in feed evaluation systems (DVE, Norfor, FiM)



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# Introduction (2)

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Method contains two steps:

1. Incubation : degradation of substrate
2. Rinsing : removal of rumen contamination

Consequence of rinsing:

Also the removal of soluble components (S) and small particles (W-S) from the feed ingredient →

Only the *in situ* degradation of insoluble nutrients in large particles can be measured (non washout or D fraction).

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# Introduction (3)

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*In situ* fractional degradation S and W-S are based on assumptions:

N

S-fraction:  $2.0 \text{ h}^{-1}$  (DVE/OEB 2011)

$1.5 \text{ h}^{-1}$  (Norfor 2011)

W-S fraction:  $k_d(D)$  (DVE/OEB 2011; Norfor 2011)

Starch :

W-S fraction:  $0.375 + 2 * k_d(D)$  (DVE/OEB 2011)

$1.5 \text{ h}^{-1}$  (Norfor 2011)

Traditional rinsing method: washing machine

Disadvantage:

1. Different machines // programs : no standardization  
Large contribution to the total variation
2. Additional determination of S fraction needed
3. W-S fraction is calculated : affected by bias between methods and no control possible

Modified rinsing method was developed:

de Jonge et al. (2013) *Animal* 7, 1289-1297

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# Aim

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1. Comparison between the non washout fractions for grass silages and maize silages obtained with both methods
2. Consequences of differences between both rinsing methods for feed evaluation (DVE/OEB 1991)
3. Prediction of the non washout fraction based on the chemical composition of the samples



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# Materials

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99 Grass silages ; fresh, cut at 1 cm

99 Maize silages ; fresh, cutter

Samples from a large *in situ* project.

Both rinsing methods: 5 g of DM was weighed into each nylon bag



Variable	Maize silage (n = 99)		Grass silage (n= 99)	
	Minimum	Maximum	Minimum	Maximum
Chemical composition (g/kg DM)				
<b>Dry matter</b> (g/kg FM)	272.2	440.4	201.0	685.0
Ash	21.0	79.0	70.0	192.0
<b>Crude protein</b>	52.6	81.0	102.0	222.0
Crude fat	27.0	47.0	27.0	65.0
<b>Starch</b>	176.0	427.0	-	-
Sugar	3.0	43.0	11.0	246.0
Neutral detergent fiber	278.0	503.0	326.0	611.0
Acid detergent fiber	152.0	289.0	157.0	347.0
Acid detergent lignin	11.0	27.0	10.0	40.0
Silage quality parameters				
pH	3.60	4.40	3.90	6.67
NH3-N (g N/kg DM)	0.33	2.30	0.93	7.94

## Standard washing machine method

- Bags were washed in a washing machine using wool program without centrifugation (40 min, tap water at 25°C) according to the method described by Tas et al. (2006).
- Nylon bags air dried at 70°C ; weighted; ground at 1 mm and analysed

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# Methods

# (2)

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## Modified rinsing method

- Two bags of each maize or grass silage were placed in a glass vessel (Ø 19 cm, 7 cm height), containing 500 ml buffer solution (pH 6.2) at room temperature.
- The glass vessel, containing the buffer solution with the bags, was placed in a mechanical water shaker (160 spm) for 1 h.
  
- Nylon bags air dried at 70°C ; weighted; ground at 1 mm and analysed



# Results

(1)

## Non washout fractions of grass silages

Fractionation method	Mean	SD	Minimum	Maximum
N				
Washing machine	0.565	0.099	0.362	0.884
Modified method	0.533	0.085	0.361	0.763



# Results

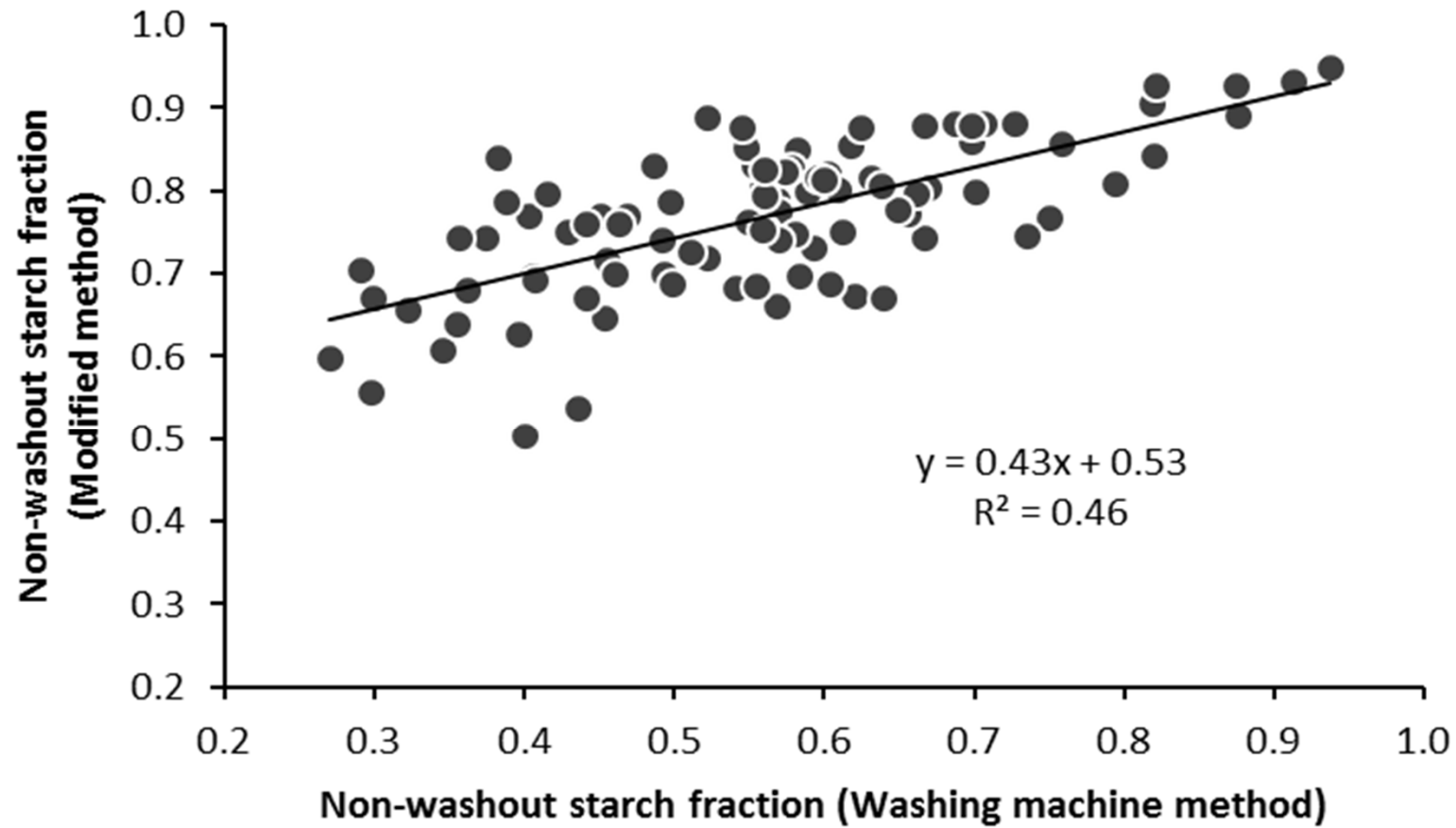
(2)

<b>Non washout fractions of maize silages</b>				
<b>Fractionation method</b>	<b>Mean</b>	<b>SD</b>	<b>Minimum</b>	<b>Maximum</b>
<b>N</b>				
<b>Washing machine</b>	<b>0.469</b>	<b>0.091</b>	<b>0.266</b>	<b>0.796</b>
<b>Modified method</b>	<b>0.491</b>	<b>0.085</b>	<b>0.335</b>	<b>0.754</b>
<b>Starch</b>				
<b>Washing machine</b>	<b>0.560</b>	<b>0.142</b>	<b>0.270</b>	<b>0.938</b>
<b>Modified method</b>	<b>0.768</b>	<b>0.089</b>	<b>0.502</b>	<b>0.948</b>



# Results

(3)



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# Effects on Feed evaluation

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For N in grass silage and maize silage:

No systematic effect of rinsing method on ED

For starch in maize silage using modified rinsing method:

D-fraction increase 0.2 (20% abs.)

ED decrease 0.05 ( $k_d = 0.112 \text{ h}^{-1}$ ;  $k_p = 0.06 \text{ h}^{-1}$ )

Intestinal available protein DVE decrease with 1.7 g/kg DM  
(344 g/kg DM starch; DVE/OEB 1991)



# Regression equations

(1)

## Nitrogen in grass silages

Regression equation

R<sup>2</sup>

RMSE

### Washing machine method

**D+U = 0.326 (± 0.042) + 0.001 (± 0.00001)**  
**DM**

**0.40**

**0.07**

### Modified method

**D+U = 0.072 (± 0.051) + 0.001 (± 0.00001)**  
**DM + 0.001 (± 0.00001) NDF**

**0.61**

**0.05**





# Regression equations

(2)

## Maize silages

Regression equation

R<sup>2</sup>

RMSE

### Washing machine method

$$D+U (N) = 0.853 (\pm 0.101) - 0.006 (\pm 0.002) \text{ CFat} + 0.001 (\pm 0.0003) \text{ NDF} - 0.002 (\pm 0.001) \text{ ADF}$$

0.23

0.08

$$D+U (\text{Starch}) = 0.471 (\pm 0.188) + 0.001 (\pm 0.0003) \text{ DM} + 0.001 (\pm 0.0004) \text{ NDF} - 0.004 (\pm 0.001) \text{ ADF}$$

0.43

0.11

### Modified method

$$D+U (N) = 0.842 (\pm 0.089) - 0.004 (\pm 0.001) \text{ CFat} - 0.001 (\pm 0.003) \text{ ADF} + 0.007 (\pm 0.003) \text{ ADL}$$

0.19

0.08

$$D+U (\text{starch}) = 0.177 (\pm 0.156) + 0.001 (\pm 0.0001) \text{ DM} - 0.003 (\pm 0.001) \text{ CP} - 0.004 (\pm 0.002) \text{ CFat} + 0.001 (\pm 0.0002) \text{ NDF} - 0.003 (\pm 0.001) \text{ ADF} + 0.008 (\pm 0.002) \text{ ADL}$$

0.54

0.06



- No systematic difference for the non washout fraction of N in grass silage and maize silage obtained with both rinsing methods were found.
- Modified rinsing method increased the non washout fraction of starch in maize silage, especially in case of a large washout fraction.

- New rinsing method decreased the calculated ED for starch and therefore the DVE value of maize silage but effect was modest: 1.7 g/kg DM  
Average DVE – value : 52 g/kg DM
- Regression analysis showed weak and moderate relationships between non washout fraction of N and starch and the chemical composition of maize and grass silages:  
**No alternative to estimate the non washout fraction**

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Thanks for your attention

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***Questions***



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