***In vitro* gas production profile of non-washable, insoluble washable and soluble washable fractions in some concentrate ingredients.**

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It is assumed that rumen degradation of material washed out of nylon bags is instantaneous and complete. Using a standardised laboratory procedure that mimics the results of washing in the *in situ* technique (Azarfar et al., 2004) along with an *in vitro* gas production technique seems to be a promising method to verify this assumption. In a 6×4 factorial arrangement of treatments with three replicates, samples of 6 concentrate ingredients (maize, barley, milo, peas, lupins and faba beans) and 4 fractions (whole: WHO, non-washout fraction: NWF, insoluble washout fraction: ISWF and soluble washout fraction: SWF) were subjected in two runs to an *in vitro* incubation technique, which measures gas production continuously in an automated system (APES) for 72 h. The Gas production profiles, obtained with APES, were fitted to a multi-phasic model (Groot et al., 1996).
The fermentation characteristics of ISWF were more similar to those of WHO and NWF than to those of SWF. Dividing the gas production profile of SWF into two phases, revealed a very rapidly degradable fraction in the first phase of degradation. Fitting the gas production profiles of WHO with a tri-phasic model revealed that the calculated sub-curve of the first phase did not match with the gas production profile of SWF.

Introduction

A widely adopted procedure to characterise degradation of feedstuffs in the rumen is the *in situ* incubation technique that assumes the washable (W) fraction to be equal to the soluble (S) fraction and that both are rapidly and completely degraded. Since recovery of the washable fraction in the *in situ* technique is impossible, verifying this assumption needs the use of alternative methods. A new method of feed fractionation by which the washable fraction can be fractionated into an insoluble washable (ISWF) and a soluble washable fraction (SWF) along with the *in vitro* gas production technique seems to be a promising method to characterise the degradative behaviour of these fractions in the feedstuffs. A gas production profile resulting from microbial degradation of feedstuffs can be characterised by several mathematical models (France et al., 2000) of which a multiphasic model described by Groot et al. (1996) seems to be very appropriate to describe the degradative behaviour of feedstuffs. Gas production profiles of most feed samples can be fitted with this model consisting of multiple phases. Each phase is characterised by the asymptotic gas production, the time lapse from incubation to the time at which half of the maximum gas is produced and a parameter determining the shape of the curve. Cone et al. (1996; 1997) showed that the first phase represents the fermentation of the soluble fraction, the second phase the non-soluble fraction and the third phase is caused by microbial turnover. Therefore, the aims of this study were:

1- To characterise the degradative behaviour of whole, NWF, ISWF and SWF in some concentrate ingredients (maize, barley, milo, peas, lupins and faba beans).

2- To verify the assumption of Cone et al. (1996; 1997) that the first and second phases represent the fermentation of the soluble fraction and non-soluble fraction.

Material and methods

In a 6×4 factorial arrangement of treatments with three replicates, samples of 6 concentrate ingredients (maize, barley, milo, peas, lupins and faba beans) and 4 fractions (whole: WHO, NWF and ISWF) in two runs were subjected to an *in vitro* incubation technique, which measures gas production continuously in an automated system (APES) for 72 h.. The whole concentrate ingredients were included in each *in vitro* incubation run as a control throughout the runs to establish possible effects of the runs on gas production. The fractionation of concentrate ingredients into NWF, ISWF and SWF is described in detail by Azarfar et al. (2004). Three replicates of each substrate were fermented in 100 ml specific APES bottles containing 0.5 g DM of each substrate, 82 ml medium B and 5 ml of rumen fluid inoculum in the automated pressure system for 72 h. Rumen fluid was a mixture obtained from three non-lactating cows, fed once daily on a diet of moderate quality grass hay ad libitum and 1 kg of commercial concentrates.

Table 1. Chemical composition of feedstuffs and fractions

		% of Total	ASH (g/kg DM)	Starch (g/kg DM)	CP (g/kg DM)	NDF (g/kg DM)	Sugar (g/kg DM)	OM Residue
Maize	Whole	100	14.1	740.2	97.1	95.6	20.2	32.7
	NWF	82.5	5.4	792.7	95.8	107.6	1.1	-2.6
	ISWF	13.1	5.3	789.6	62.8	20.1	4.6	117.6
	SWF	4.4	186.5	5.2	136.2	ND	337.3	334.7
Barley	Whole	100	24.7	586.4	133.8	167.7	29.8	57.5
	NWF	76.7	19.4	587.6	134.5	199.4	9.6	49.6
	ISWF	18	6.5	839.8	83.6	17.4	2.3	50.7
	SWF	5.3	154.5	6.2	158.0	ND	332.3	349.1
Milo	Whole	100	19.9	746.9	119.3	75.3	9.3	29.2
	NWF	70.2	10.2	766.7	142.3	94.7	0.5	-14.4
	ISWF	24.3	6.8	925.8	40.0	19.6	0.7	7.1
	SWF	5.5	199.8	19.2	190.2	ND	136.8	454.0
Peas	Whole	100	33.0	490.7	237.5	94.5	56.4	87.9
	NWF	41.3	22.0	520.4	159.9	250.5	17.4	29.8
	ISWF	37	12.2	838.8	152.0	24.3	11.3	-38.7
	SWF	21.7	92.0	1.6	503.5	ND	259.7	143.1
Lupins	Whole	100	32.5	9.5	361.0	260.1	61.0	275.8
	NWF	70.4	21.2	6.3	297.0	367.0	24.0	284.5
	ISWF	13.6	19.7	5.5	402.0	296.0	17.5	259.2
	SWF	16	87.7	0.9	270.4	ND	288.9	352.1
Faba beans	Whole	100	32.0	407.8	340.2	121.9	39.4	58.8
	NWF	43.2	20.8	399.5	236.3	309.2	12.7	21.6
	ISWF	37.9	10.4	503.8	274.8	207.0	8.6	-4.6
	SWF	18.9	96.1	0.7	606.5	ND	174.3	122.3

The sample of rumen fluid for the inoculum was taken at 08.00 h in the morning, prior to feeding. Gas production profiles, obtained with the automated system, were fitted to a multi-phasic model as described by Groot et al. (1996) as shown in the equation below:

$$G_t = \sum_{i=1}^{n} \frac{A_i}{1 + (C_i / t)^{Bi}}$$

where i is the number of phases, G_t is the cumulative gas production at time t (ml/g OM incubated), A_i is the estimated asymptotic gas production (ml/g OM incubated) at time t, B_i represents the sharpness of the switching characteristic for the profile and C_i is the time (h) of incubation at which half of the asymptotic gas production has been formed.

Results

The chemical composition of the feedstuffs and their fractions is shown in table 1. The results show that SWF is relatively rich in ash, crude protein, soluble sugars and a residual unknown fraction. Except in lupins, the ISWF fraction is relatively rich in starch. The gas production characteristics of concentrate ingredients after 72 h incubation in APES are shown in Table 2.

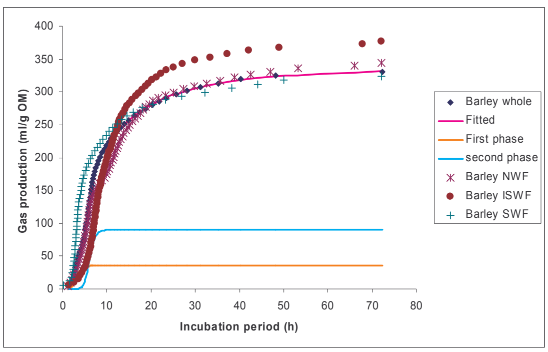
Table 2. Cumulative gas production (OMCV, ml g^{-1} OM), asymptotic gas production (A1 and A2, ml g^{-1} OM), half time of gas production (C1 and C2, h) and maximum rate of substrate degradation (RmD1 and RmD2, h^{-1}) in some concentrate ingredients after 72 h incubation.

Item	Grain						Fraction			
	Maize	Barley	Milo	Peas	Lupins	Faba beans	WHO	NWF	ISWF	SWF
OMCV	326.6^{b}	327.5^{b}	308.4^{c}	351.1^{a}	281.4^{d}	314.7^{c}	326.5^{b}	339.3^{a}	335.1^{a}	268.2^{c}
A1	112.0^{a}	126.9^{b}	104.3^{b}	108.6^{a}	135.5^{b}	175.2^{b}	140.5^{a}	154.9^{a}	131.8^{a}	122.2^{a}
A2	226.0^{a}	203.6^{ab}	215.6^{ab}	179.9^{cd}	156.6^{c}	151.3^{c}	194.9^{ab}	191.9^{ab}	207.6^{a}	164.8^{b}
C1	7.5^{b}	5.6^{c}	7.9^{b}	8.0^{b}	5.7^{c}	8.9^{a}	7.8^{c}	8.5^{b}	9.3^{a}	5.0^{b}
C2	14.0^{c}	10.0^{d}	13.0^{c}	18.1^{b}	21.3^{a}	19.6^{ab}	14.9^{b}	16.9^{a}	15.8^{ab}	16.5^{ab}
RmD1	0.85^{a}	0.87^{a}	0.66^{ab}	0.45^{cb}	0.35^{cd}	0.18^{d}	0.38^{b}	0.5^{b}	0.5^{b}	1.09^{a}
RmD2	0.014^{c}	0.187^{a}	0.018^{c}	0.137^{ab}	0.06^{cb}	0.113^{ab}	0.153^{a}	0.071^{b}	0.028^{b}	0.022^{b}

^{abcd} Means with different superscript within a row differ significantly (*P<0.05*).

Statistical analysis showed that the fermentation characteristics of ISWF were more similar to those of WHO and NWF than to those of SWF. Total gas production of SWF was considerably lower than that of WHO, NWF and ISWF. The relatively high crude protein content is kept responsible for this, because it is known that gas yield of the degradation of protein is lower than that of carbohydrates like starch and NDF. The gas production profile of SWF showed two phases, the first of which represented a very rapidly degraded fraction, of which the half time was reached much earlier than that of the first phase of WHO, NWF and ISWF. However, the data do not support the assumption that SWF is instantaneously degraded.

Cumulative gas production of some concentrate ingredients, their fractions and the calculated sub-curves of phase 1 and phase 2 are shown in Fig 1. The calculated sub-curves 1 and 2 did not match with the gas production profiles of SWF, ISWF and NWF.



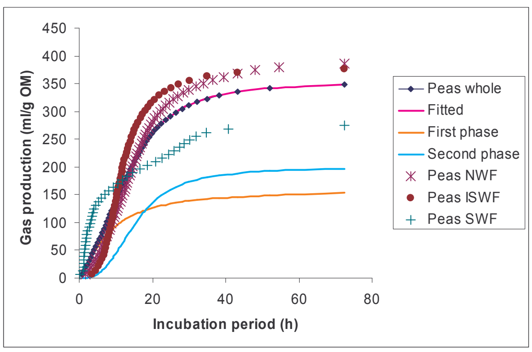


Fig. 1. Cumulative gas production profile of barley and peas, their fractions and calculated sub-curves of phase 1 and 2.

Conclusion

Our data show that the size of the soluble fraction is unequal to that of the washout fraction. Furthermore, the assumption that the first phase represents the fermentation of the soluble fraction and the second phase that of the non-soluble fraction can not be confirmed by our data.

References

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