Effects of washing procedure, grain type and particle size on the size of non-washout and insoluble washout fractions in concentrate ingredients.

A. Azarfar[1], S. Tamminga[1] and H. Boer[1]*
[1]Wageningen University, Animal Nutrition Group, P. O. Box 338, 6700 AA Wageningen, The Netherlands

Abstract
It is assumed that rumen degradation of material washed out of nylon bags is instantaneous and complete. Verifying this assumption requires a standardised laboratory procedure that mimics the results of washing in the *in situ* technique. In a 2x3x6 factorial design of treatments we compared the effects of three washing procedures (Y=Yang, M=Melin, I=*In situ*) in six grains (maize, barley, milo, peas, lupins and faba beans), ground at two particle sizes (1 and 3 mm), on the size of the non-washout (NWF) and the insoluble washout fraction (ISWF). Differences between methods were washing in a washing machine (I), repeated mechanically driven plunging of nylon bags in a beaker (Y) and filtration according to the procedure of Melin et al (2001) (M).
Grain type, washing method and particles size had significant (*P<0.05)* effects on NWF and ISWF. NWF in method Y was smaller than in the other methods (53.4, 58.0 and 60.4% in Y, M and I, respectively). Method M was more similar to method I. NWF in maize, barley, milo, lupins, faba beans and peas was 77.5, 68.1, 61.3, 65.9, 37.8 and 33.5% respectively. ISWF in peas, faba beans, milo, barley, maize and lupins was 40.9, 37.3, 32.7, 24.8, 15.5 and 13%. Increasing the particle size increased NWF whereas the size of ISWF was decreased.

Introduction

A widely adopted procedure to characterise degradation in the rumen is the *in situ* incubation technique, that assumes the washable (W) to be equal to the soluble (S) fraction and that this fraction is rapidly and completely degraded. This assumption has been challenged (Lopez et al., 1994; De Boever et al., 1995; Madsen et al., 1995). A drawback of the method is that recovery of the washout fraction is not possible; therefore, alternative fractionation methods should be developed in order to recover and fractionate the washout fraction for further studies and analysis. Recently, two simple fractionation methods have been developed by which the washout fraction can be separated into an ISWF and a soluble washout fraction (SWF) (Yang et al., unpublished; Melin et al., 2001). The aims of this study were:

1- To choose an appropriate washing procedure to mimic the washing machine procedure.
2- To study the effects of washing procedure (Methods I, M and Y), grain types (maize, barley, milo, peas, lupins and faba bean) and particle size (1 and 3mm) on the size of NWF and ISWF.

Material and Methods

In a 3x6x2 factorial arrangement of treatments with 3 replicates, the effects of 3 different washing procedures (IS, M and Y) on the size of NWF and ISWF in 6 concentrate ingredients (maize, barley, milo, peas, lupins, faba beans) with 2 particle sizes (1 and 3 mm) were studied. Washing of the samples with the washing machine was according to the CVB protocol (CVB, 2003). In method Y a 100 g sample of a ground concentrate ingredient (1 and 3mm) was weighed into a nylon bag (Nybolt PA 40/30), with an effective size of 16×23 cm, that was subsequently placed in a 3000 ml

volume beaker (27 cm height) filled with 1000 ml of de-mineralized water at room temperature. To mimic the standard washing procedure of the nylon bag incubation technique, the bag was frequently lifted above and submerged into the water for 1 h by an excentric rod driven by a stirring motor at a speed of 40 rpm. After removal of the bag with its residual contents, the liquid mixture in the beaker was centrifuged at 8000 rpm for 20 min in a Beckman 2-21M centrifuge, to mimic filtration. The washing and centrifugation procedure was repeated twice with pouring another 1000 ml of demineralised water into the beaker. After each centrifugation the pellet left inside the centrifuge tubes was collected into a pre-weighed aluminum container, and some water was used to get the residual pellet into the same container for freeze-drying, which resulted in the ISWF. The NWF, i.e., the residue left inside the nylon bag, was collected and also freeze-dried.

In method M, 3 g of each sample was weighed into a 250-ml beaker and soaked in 25 ml of distilled water at a constant shaking rate of 400 rpm/min for 1 h. After soaking, the samples were poured into a funnel with a filter (a piece of nylon bag cloth 20x20 cm, Nybolt PA 40/30) that was fixed into the funnel's brim with paper clips. Beakers were washed twice with 10 ml of distilled water to get the remaining feed into the filters. The samples in the filter were firmly stirred with a spatula until water passed through the filters. Then, samples were rinsed with 10 ml of distilled water. Rinsing was repeated twice. After the last rinsing, samples were left to drip for 15 minutes to ensure that all rinsing water had passed the filer. Filters and the residue inside the filter were dried at 70 ^{0}C for at least 12 hours. The residue inside the filter is assumed to be NWF. The resulting filtrate was centrifuged like in Method Y and the pellet left inside the centrifuge beakers was collected with the use of some water into pre-weighed aluminum containers for freeze-drying (ISWF).

Results

As shown in table 1, washing procedure, grain type and particle size had significant effects (*P<0.05)* on the size of NWF and ISWF. The M method gave results fairly close to those derived with the standard machine washing. Of the grains, the peas and faba beans, both legume seeds, resulted in a considerably lower NWF than the other grains. This is probably because in these two seeds a lot of protein was washed out. As could be expected, reducing the particle size from 3 to 1 mm caused a larger residue that could not be washed out.

Table 1. Effects of washing procedure grain types, and particles size on the size of non-washout and insoluble washout fractions.

Item	Washing Method			Grain Types[1]						Particles size (mm)	
	I	M	Y	1	2	3	4	5	6	1	3
NWF%	**60.4^{a}**	**58.0^{b}**	**53.5^{c}**	**77.5^{a}**	**68.1^{b}**	**61.3^{c}**	**33.5^{d}**	**65.9^{e}**	**37.8^{f}**	**54.4^{a}**	**60.3^{b}**
ISWF%	**ND**	**25.6^{b}**	**29.1^{b}**	**15.5^{a}**	**24.8^{b}**	**32.7^{c}**	**40.9^{d}**	**13.0^{e}**	**37.3^{f}**	**29.9^{a}**	**24.9^{b}**

● Different superscripts indicate significant differences (*P < 0.05).*

[1] 1, 2, 3, 4, 5, and 6 are Maize, Barley, Milo, Peas, Lupins and Faba bean, respectively.

ND: not determined.

Conclusion

It is concluded that method M was more appropriate than method Y to mimic the washing machine procedure due to ease of handling and slightly higher correlation

with the results of the washing machine procedure (R^2=0.9563 vs 0.9461 in methods M and Y, respectively).

Literature

CVB, C. V. B. 2003. Voorloping protocol voor *In situ* pensincubatie voor het meten van eiwitbestendigheide, 2003.

De Boever, J. L., V. J. M., D. F. Bogaerts, and C. V. Boucque. 1995. Protein evaluation of cattle compound feeds: Comparison of *in sacco* measurements and tabular values. Neth. J. Agric. Sci. 43: 297-311.

Lopez, S., J. France, and M. S. Dhanoa. 1994. A correction for particular matter loss when applying the polyester bag method. Br. J. Nutr. 71: 135-137.

Madsen, J. et al. 1995. The AAT/PBV protein evaluation system for ruminants. A revision. Norw. J. Agri. Sci. (Suppl. 19): 1-37.

Melin, M., M. Gierus, G. A. L. Meijer, and A. M. van Vuuren. 2001. Chemical characteristics and degradability of crude protein in the S-fraction of feedstuffs. ID-Lelystad. NO: 2079.

Yang, H.-J., S. Tamminga, B. A. Williams, J. Dijkstra, and H. Boer. 2000. In vitro gas and VFA production profiles of barley and corn and their soluble and washout fractions after feed processing. Unpublished data.