Influence of soluble content losses on the ability of aerobic fungi to improve the nutritional content of pre-treated forages

^{a*}Idowu, O. J., ^bChaudhry, A. S., ^cDolfing, J. and ^aOjo, V. O. A.

^aDepartment of Pasture and Range Management, Federal University of Agriculture,
P.M.B. 2240, Abeokuta, Nigeria

^bSchool of Natural and Environmental Sciences,

School of Civil Engineering and Geosciences, Newcastle University, NE1 7RU,
Newcastle upon Tyne, United Kingdom

Corresponding author: idowuoj@funaab.edu.ng or o.j.bolaji@newcastle.ac.uk

Abstract

Fungal improvement of the nutritive content of low-quality forages can be affected by several factors among which loss of water-soluble content (WSC) plays a major role. To achieve this aim, two growth conditions i.e. forage-liquid ratios (1:3 and 1:5) and two inoculation times (14 and 28 days) were used to cultivate the selected fungi i.e. Pleurotus ostreatus (PO) at 30^oC and Ceriporiopsis rivulosus (CR) at 20^oC on Brachiaria decumbens (BD), Andropogon gayanus (AG), Triticum aestivum (TA) straw, Lolium perenne (LP), respectively with the view of selecting the optimal conditions that facilitate the release of WSC. The impact that losses of WSC have on the ability of fungi to improve the nutritive content (i.e. proximate, fibre, secondary metabolites and total antioxidant content) of the forages were then measured using 2 filtering methods i.e. light pump filtering method (LFM) and free flow filtering method (FFM). The optimal conditions that supported increased soluble was identified as 28th day for both fungi; forage-liquid ratio of 1:5 for both fungi in most of the forages except BD (CR) and BD & AG (PO). The LFM led to lesser or no improvement in the nutritive quality of the upgraded forages while the opposite was recorded with the FFM. The LFM as against the FFM produced upgraded forages with lesser reduction in NDF; similar or higher ADF and lignin contents; and similar reduction in secondary metabolites and TAC when compared with un-improved forages. It can be concluded that the fungal improvement of the nutritive content of low-quality forages was negatively affected by the loss of WSC. It is therefore recommended that fungal improvement of the nutritive quality of low quality forages should be carried out with methods or techniques that facilitates little or no WSC loss in the upgraded forages.

Keywords: Water soluble content, fungal improvement, inoculation time, forage-liquid

Introduction

Available forages, during the dry season in most tropical regions are of lower quality and need to be improved upon to be properly utilised by ruminant animals for better production and performance. One of the possible ways of achieving this is through fungal treatment. During fungal treatment of low quality forages, lignocellulose degradation takes place and this is usually accompanied with the release of WSC. The WSC are by-products of the degradation process and they are released when the conditions are optimal for growth and enzymatic function. Despite the need

for the growth conditions to be optimal for improvement, in some cases, no improvements were achieved. This led some researchers to question the ability of these fungi in improving the pre-treated substrates. This un-improvement have been traced to several factors such as substrate type (van Kuijk *et al.*, 2015), fungal strain and species, extent of degradation (Akinfemi, 2010) and release of anti-nutrients as toxins or metabolites by these fungi which affect palatability (Sharma and Arora, 2015), chitin content (Arora and Sharma, 2009a) and extensive fermentation of structural carbohydrates with increased

Soluble content losses impact on ability of fungi to improve pre-treated forages

ash content (Sharma and Arora, 2015). Most of these factors do have a major influence on the nutritive content, intake, digestibility and/or degradability of the pre-treated substrates (Mahesh and Mohini, 2013). However, no information is available on the impact that WSC loss may have on the nutritive quality of fungal pre-treated substrates. In order to enable the liberation of WSC, two growth conditions i.e. substrate-liquid ratios (1:3 and 1:5) and inoculation time (14 and 28 days) that play major roles in fungal growth and activity were selected in this study. Thereafter, the optimal conditions was then used to evaluate the influence that loss of WSC have on the ability of two fungi (i.e. *C. rivulosus*; and *P. ostreatus*) selected based on their use and higher activity to improve the nutritive content (i.e. proximate, fibre, lignin secondary metabolites and total antioxidant content-TAC) of the selected low quality forages (i.e. *A. gayanus*, *B. decumbens*, *L. perenne* and *T. aestivum* straw) using light-pump filtering method (LFM) and free-flow filtering method (FFM) at the optimal growth conditions.

Materials and methods

Preparation of forage substrates and inoculation with aerobic fungi

A 4 (forages i.e. AG, BD, TA, and LP) x 2 (substrate: liquid ratio i.e. 1:3 and 1:5) x 3 (fungal treatment i.e. CR, PO and no fungi) factorial arrangement at each inoculation time (14 and 28 days) were used for the growth and laccase study, and each treatment was replicated thrice. The samples consisted of the two Nigerian low-quality forages (*BD* and *AG*) and two UK forages (*TA* straw and *LP*). On this basis, samples of 5g per forage (2-mm screen) were accurately weighed into 250 mL Erlenmeyer flasks, and were rehydrated with two different amounts (15 and 25 mL) of 1% Potato dextrose broth (CR) and 1% malt extract broth (PO) respectively to set the solid/liquid ratio of 1:3 and 1:5. The flasks were immediately covered and

autoclaved at 121°C for 15mins. Each of the Erlenmeyer flasks was then inoculated with three 5mm mycelial discs of each agar plugs of CR and PO obtained from a 10 days old plate culture at the centre of the substrate respectively, covered and incubated at either 20°C (CR) or 30°C (PO) in a dark environment at 95% humidity for 14 and 28 days, respectively. Replicated control flasks containing the substrates and the media but no fungal mycelium were also autoclaved.

Processing of inoculated substrates for growth and laccase enzymatic activity

At the end of each selected inoculation time, the samples assigned for each filtering method obtained from the various combinations of growth conditions were physically viewed for growth and supernatants obtained from each filtering method was used for laccase analysis. The supernatant was obtained by adding 25mL of 10mM sodium acetic buffer solution (pH 5.0) to each selected flask for extraction of enzymes. The flasks were then kept in a shaking incubator (200rpm) for 20mins at 20°C. The contents of each flask were filtered through a dried what-man filter paper no. 1 (150 mm diameter) and the filtrate was centrifuged at 10,000 rpm at 4°C for 20 min. The supernatant was collected for its use for laccase enzyme determination (Sharma and Arora, 2010). Laccase activity was determined by monitoring the oxidation of the diammonium salt of ABTS (2, 2' - azino - bis (3 - ethylbenz - thiazoline - 6 - sulfonic acid) as a substrate. (Geng *et al.*, 2004)

Processing of the substrates for the evaluation of loss of water-soluble contents

A 4 (forages i.e. AG, BD, TA, and LP) x 3 (fungal treatment i.e. CR, PO and no fungi) factorial arrangement was used for evaluation of loss of WSC. Only the best inoculation time (i.e. 28 days) and forage-liquid ratio i.e. 1:5 for both CR and PO for most of the forages except BD (CR) and BD & AG (PO) was used. These conditions should optimal fungal growth and laccase activity and was used for each combination. The filtrates were collected and the flasks

from which the solids on the filter paper were obtained were then split equally to two different parts. Half of the flasks with their solids on the filter paper were used as samples obtained from free-flow filtering method under gravity (i.e. FFM) without any pressure, while the other half of the flasks with their solids on the filter paper were used as samples obtained from light pump filtering method with light suctioning using pump (i.e. LFM). The contents of each filtering method, alongside the filter papers, were then dried at 60°C and subsequently used for various analyses, i.e. chemical analysis, secondary metabolites and total antioxidant content analyses. Loss of dry matter and other nutrients as influenced by fungal inoculation was calculated as the differences in absolute weight between the control (un-inoculated substrate) and the inoculated substrates and the results were presented as percentages of the relevant nutrients of the untreated controls.

Determination of the nutritive contents of the dried fungal degraded samples obtained from LFM or FFM

The samples for each filtration method were analysed in triplicate for dry matter (DM) and Organic matter (OM) using methods. Nitrogen values were obtained using an Elementar vario macro cube (Elementar, Hanau Germany). The crude protein (CP) content was then calculated ($N \times 6.25$). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin were assessed using the methods of and without using sodium sulphite and Dekalin. Total phenolic (TP) and total tannin (TT) contents were measured using modified Folin-Ciocalteu method. Both TP and TT were expressed as tannic acid equivalents. TAC of the samples were determined according to the Ferric Reducing Antioxidant Power assay and results obtained were then expressed as $\mu\text{mol Fe (II)}/\text{g dry weight of the sample}$.

Statistical analysis

The data for laccase activity were statistically analysed by following the factorial design according to the Generalized Linear Model procedure in Minitab 16 software to determine the interactive effects of each fungal treatment, forage and forage: liquid ratio on the laccase activity of the fungi at each of the inoculation times (14 and 28 days). The data for chemical composition were statistically analysed by following the factorial design according to the Generalized Linear Model procedure in Minitab 16 software to determine the influence of filtration method (i.e. LFM and FFM) on the interactive effects of fungal strain and forage at the optimal forage-liquid ratio and inoculation time on the chemical composition and nutrient losses. $P < 0.05$ was considered to indicate a statistically significant effect on each of the tested parameters and different means were separated using Tukey's HSD option of the software.

Results

Growth and laccase activity

Figure 1 and 2 shows the main effect of forage-liquid ratio at each inoculation time on laccase production by CR and PO respectively on the selected forages after 14th and 28th days of inoculation respectively. The highest laccase activity produced by *C. rivulosus* was at 1:5 ratio with the exception of BD at 28th day. The highest laccase activity produced by *P. ostreatus* for BD and AG was at 1:3 ratio; that of LP recorded similar value for both forage-liquid ratios; and that of TA was at 1:5 ratio at the 28th day. Generally, PO recorded better growth and activity on most of the forages except with LP where CR was found actively growing on it. The growth of the fungi was viewed physically and it was found to be similar in trend to the activity that was why it was not recorded.

Soluble content losses impact on ability of fungi to improve pre-treated forages

Figure 1. The interactive effect of forage-liquid ratio and forage on the laccase activity (μmol / min) of fungi after 14 days of inoculation

Figure 2. The interactive effect of forage-liquid ratio and forage on the laccase activity (μmol / min) of fungi after 28 days of inoculation

Nutritive contents and nutrient loss /gain (Proximate, fibre, lignin, secondary metabolites and total antioxidant compositions) of fungal pre-treated forages as influenced by the filtration methods

Table 1 presents the influence the filtration methods (i.e. LFM and FFM) produced on the interactive effects that fungus and forage had on the proximate, fibre, lignin and secondary metabolites composition of pre-treated forages. The filtration methods had significant ($P < 0.05$) influence, in that the LFM method led to the inability of both fungi to rapidly increase CP; rapidly reduce OM and increase Ash; facilitate increased reduction in NDF; lesser reduction or no reduction in ADF and ADL; and rapidly reduce TAC and secondary metabolites content of most of the pre-treated forages when compared to the impact the FFM method had on the pre-treated forages with

respect in both cases to the untreated forages. More pronounced negative effect of the LFM method on the ability of PO to improve most of the forages except LP was recorded, as its activeness and growth led to more availability of WSC which was further lost through the method thus leading to lesser impact of the fungus on the forage improvement when compared to the less active fungus.

Table 2 presents the influence that filtration methods (i.e. LFM and FFM) produced on the interactive effects of fungi and forage on the gross nutrient loss of the pre-treated forages. The pre-treated forages from the LFM method recorded less loss in OM, fibre and lignin contents as well as less gain in CP and ash; lesser loss in NDF; lesser loss or no loss in ADF; and lesser TP, TT and TAC losses than those obtained from the FFM method with respect to the untreated forages at both cases.

Table 1: The interactive effects of forage and forage:liquid ratio on the proximate, fibre, and secondary metabolite compositions (g/kg DM) of 28 days pre-treated forages obtained from light pump filtering and free flow filtering methods

Par	<i>A. gayanus</i>			<i>B. decumbens</i>			<i>L. perenne</i>			<i>T. aestivum</i>		
	Con	CR/1:5	PO/1:3	Con	CR/1:3	PO/1:3	Con	CR/1:5	PO/1:5	Con	CR/1:5	PO/1:5
Proximate composition												
OM(L)	956.43 ^{ab}	950.16 ^{bc}	935.92 ^{ef}	949.44 ^{bcd}	943.87 ^{cde}	929.56 ^f	959.70 ^a	943.37 ^{cde}	920.90 ^g	941.73 ^{de}	940.67 ^e	930.49 ^f
OM (F)	967.55 ^a	938.02 ^e	919.72 ^f	960.66 ^b	949.89 ^{cd}	937.17 ^e	953.69 ^c	916.99 ^f	900.67 ^g	968.70 ^a	951.10 ^{cd}	947.90 ^d
Ash (L)	43.58 ^{fg}	49.37 ^{ef}	64.09 ^{bc}	50.56 ^{def}	56.14 ^{cde}	70.44 ^b	40.30 ^g	56.63 ^{cde}	79.10 ^a	58.27 ^{cd}	59.33 ^c	69.51 ^b
Ash (F)	32.45 ^g	61.98 ^c	80.28 ^b	39.34 ^f	50.11 ^{de}	62.83 ^c	46.31 ^e	83.02 ^b	99.33 ^a	31.30 ^g	48.90 ^{de}	52.10 ^d
CP (L)	119.96 ^d	134.45 ^e	142.77 ^e	61.13 ^e	62.16 ^e	63.52 ^e	156.80 ^b	183.06 ^d	182.56 ^a	27.64 ^f	34.20 ^f	32.66 ^f
CP (F)	120.17 ^d	159.96 ^c	180.71 ^b	58.16 ^g	71.59 ^f	76.53 ^e	156.01 ^c	177.41 ^b	187.05 ^a	33.93 ⁱ	35.14 ⁱ	40.78 ^h
Fibre fraction composition												
NDF (L)	843.21 ^{cd}	839.58 ^d	820.48 ^e	860.22 ^b	848.54 ^e	836.63 ^d	761.03 ^f	735.40 ^g	732.12 ^g	868.52 ^a	861.36 ^b	861.72 ^{ab}
NDF(F)	806.29 ^c	757.43 ^e	718.07 ^g	828.52 ^b	790.28 ^d	747.05 ^f	562.63 ^h	486.68 ⁱ	503.97 ⁱ	874.09 ^a	827.29 ^b	831.51 ^b
ADF (L)	729.45 ^d	739.40 ^c	728.34 ^d	738.33 ^c	740.50 ^c	738.32 ^c	635.47 ^e	635.09 ^e	626.41 ^f	754.74 ^b	767.72 ^a	772.35 ^a
ADF(F)	538.26 ^c	510.56 ^e	487.24 ^g	546.87 ^b	521.16 ^d	501.70 ^f	425.17 ^h	384.78 ⁱ	374.44 ⁱ	572.13 ^a	538.41 ^e	540.98 ^c
ADL (L)	129.05 ^d	131.44 ^c	128.78 ^d	131.18 ^e	131.70 ^c	131.18 ^e	106.49 ^e	106.40 ^e	104.32 ^f	135.12 ^b	138.23 ^a	139.34 ^a
ADL(F)	83.16 ^c	76.52 ^e	70.92 ^g	85.23 ^b	79.06 ^d	74.39 ^f	56.02 ^h	46.33 ⁱ	43.85 ^j	91.29 ^a	83.20 ^c	83.82 ^c
Secondary metabolites and total antioxidant composition												
TP (L)	11.14 ^a	5.48 ^{de}	4.30 ^{gh}	5.94 ^d	5.04 ^{ef}	4.15 ^{gh}	8.52 ^b	3.92 ^h	4.39 ^{gh}	6.93 ^c	5.82 ^d	4.78 ^{efg}
TP (F)	17.52 ^a	6.10 ^d	5.20 ^d	10.66 ^b	5.91 ^d	5.04 ^d	10.99 ^b	5.82 ^d	7.68 ^c	9.01 ^c	5.17 ^d	5.81 ^d
TT (L)	5.72 ^a	2.34 ^{cd}	1.44 ^f	2.29 ^{cd}	1.89 ^{def}	1.50 ^{ef}	3.70 ^b	2.08 ^{cde}	1.79 ^{def}	3.51 ^b	2.59 ^c	2.27 ^{cd}
TT (F)	10.72 ^a	2.52 ^{ef}	1.58 ^f	5.15 ^{bc}	2.32 ^{ef}	1.52 ^f	5.38 ^b	2.17 ^{ef}	3.07 ^{de}	3.95 ^{cd}	2.20 ^{ef}	2.35 ^{ef}
TAC (L)	8.47 ^a	3.87 ^e	2.74 ^{fg}	5.43 ^c	4.01 ^e	2.90 ^{fg}	7.50 ^b	2.28 ^h	2.50 ^{gh}	4.81 ^d	4.12 ^e	3.04 ^f
TAC(F)	11.59 ^b	2.41 ^g	2.30 ^g	10.91 ^c	3.15 ^{ef}	2.28 ^g	14.91 ^a	2.31 ^g	3.40 ^e	6.43 ^d	2.79 ^{fg}	2.55 ^g

Means with different letters in the same column are significantly ($P < 0.05$) different, Con; control, CR; *C. rivulatus*, PO; *P. ostreatus*, SEM; standard error of means, OM; organic matter, CP; crude protein, NDF; neutral detergent, ADF; Acid detergent fibre, ADL; acid detergent lignin, TP; total p henols, TT; total tannins, TAC; total antioxidant content, L; light pump filtering method, F; free flow filtering method

Table 2: The interactive effect of forage and forage:liquid ratio on the proximate, fibre and secondary metabolite constituents' loss/gain (%) of 28 days pre-treated forages obtained from the light pump filtering and free flow filtering methods.

Parameters	<i>A. Gayanus</i>		<i>B. decumbens</i>		<i>L. perenne</i>		<i>T. aestivum</i>		SEM
	<i>C. rivulosus</i>	<i>P. ostreatus</i>	<i>C. rivulosus</i>	<i>P. ostreatus</i>	<i>C. rivulosus</i>	<i>P. ostreatus</i>	<i>C. rivulosus</i>	<i>P. ostreatus</i>	
Proximate composition loss / gain (%)									
OM(L)	0.48 ^{de}	2.27 ^b	0.59 ^{de}	2.09 ^{bc}	1.70 ^{bc}	4.04 ^a	0.11 ^c	1.19 ^{cd}	0.31
OM (F)	3.15 ^d	4.85 ^b	1.12 ^f	2.45 ^e	3.85 ^c	5.56 ^a	1.82 ^c	2.15 ^e	0.24
Ash (L)	-10.14 ^a	-51.44 ^c	-11.02 ^a	-39.32 ^{bc}	-40.51 ^{bc}	-96.26 ^d	-1.82 ^a	-19.30 ^{ab}	6.59
Ash (F)	-96.61 ^d	-140.51 ^f	-27.36 ^a	-59.69 ^b	-79.25 ^c	-114.49 ^e	-56.21 ^b	-66.42 ^{bc}	6.44
CP (L)	-12.47 ^{ab}	-18.62 ^b	-1.69 ^a	-3.91 ^a	-16.74 ^b	-16.42 ^b	-23.75 ^b	-18.19 ^b	3.50
CP (F)	-31.01 ^d	-52.58 ^e	-23.09 ^c	-31.59 ^d	-13.71 ^b	-19.90 ^c	-3.57 ^a	-20.20 ^c	1.83
Fibre fractions composition loss / gain (%)									
NDF (L)	0.57 ^c	2.56 ^b	1.36 ^c	2.74 ^b	3.37 ^{ab}	3.80 ^a	0.82 ^c	0.78 ^c	0.28
NDF(F)	6.33 ^d	10.68 ^b	4.62 ^f	9.83 ^c	13.50 ^a	10.43 ^{bc}	5.35 ^c	4.87 ^{ef}	0.28
ADF (L)	-1.56 ^c	0.34 ^b	-0.29 ^b	0.01 ^b	0.06 ^b	1.43 ^a	-1.72 ^c	-2.33 ^c	0.33
ADF(F)	5.37 ^{de}	9.26 ^b	4.70 ^c	8.26 ^c	9.50 ^b	11.93 ^a	5.89 ^d	5.44 ^{de}	0.38
ADL (L)	-2.12 ^{bcd}	0.46 ^b	-0.40 ^b	0.00 ^b	0.09 ^b	2.04 ^a	-2.31 ^c	-3.13 ^c	0.45
ADL(F)	8.33 ^{ef}	14.40 ^c	7.24 ^f	12.72 ^d	17.31 ^b	21.73 ^a	8.86 ^c	8.19 ^{ef}	0.62
Secondary metabolites and total antioxidant composition loss / gain (%)									
TP (L)	49.19 ^B	57.48 ^a	17.12 ^d	30.20 ^e	53.93 ^{ab}	48.49 ^b	16.65 ^d	31.75 ^c	2.09
TP (F)	63.06 ^{ab}	72.20 ^a	44.53 ^{cd}	52.74 ^{bc}	47.04 ^{cd}	30.17 ^e	42.59 ^{cde}	35.57 ^{de}	4.86
TT (L)	57.24 ^b	73.13 ^a	17.58 ^f	34.69 ^{de}	43.75 ^{cd}	51.62 ^{bc}	26.29 ^{ef}	35.22 ^{de}	2.95
TT (F)	74.47 ^{ab}	86.57 ^a	54.98 ^{bc}	70.52 ^{ab}	59.63 ^{bc}	43.05 ^c	44.26 ^c	40.28 ^c	9.76
TAC (L)	56.55 ^b	66.35 ^a	25.20 ^e	46.55 ^c	69.32 ^a	66.20 ^a	11.72 ^f	35.61 ^d	2.39
TAC(F)	77.99 ^b	81.22 ^{ab}	71.10 ^c	79.09 ^b	84.50 ^a	77.18 ^b	56.60 ^d	60.37 ^d	1.63

Means with different letters in the same column are significantly ($P < 0.05$) different, Con; control, CR; *C. rivulosus*, PO; *P. ostreatus*, SEM; standard error of means, OM; organic matter, CP; crude protein, NDF; neutral detergent, ADF; Acid detergent fibre, ADL; acid de detergent lignin, TP; total phenols, TT; total tannins, TAC; total antioxidant content, L; light pump filtering method, F; free flow filtering method.

Discussion

This study investigated the influence that loss of WSC have on the ability of fungi to improve nutrient composition of pre-treated forages using LFM and FFM methods. In the practical sense, the loss of WSC happens when farmers use sacks/bags/containers with pores for pre-treatment and in some instances place heavy stones on these containers which further mounts pressure on the pre-treated substrates. The means of soluble losses were then mimicked in the laboratory using two filtration methods (i.e. LFM and FFM). However, to facilitate the release of WSC in the pre-treated forages so as to achieve the aim of this study, two treatment factors, i.e. forage-liquid ratio and incubation time were used. In this study, it can be deduced that the growth conditions that supports optimal growth and activity led to availability of WSC.

Fungal growth performance and laccase activity influence on soluble availability

Fungal growth and activity on forages are expected to help facilitate the release of WSC when the growth conditions are optimal. This is because the WSC are products of increased fungal growth and activity in anticipation for degradation of structural components. The increased laccase activity and growth of *C. rivulosus* on most the forages except BD, as well as *P. ostreatus* on some forages except AG and BD, when using a substrate-liquid ratio of 1:5 at longer inoculation time (i.e. 28 days) was an indication that these conditions were optimal for their growth and activity. Optimal moisture content has been reported to positively influence fungal growth and laccase production by preventing substrate porosity or agglomeration, thus allowing oxygen transfer that is required for the exhibition of fungal ligninolytic activities (Patel *et al.*, 2009). The longer inoculation needed by the fungi to exhibit their growth and activity on the forages can be traced to the inherent characteristics of the forages as they play a significant role in the time at which the fungi gets the required nutrients needed to initiate their growth and activity (Daniel, 2016). Therefore, at these conditions of growth, it is expected that the

substrates contain more WSC. This makes the use of pre-treated substrates in this liquid ratio and inoculation time a good choice for the chemical composition determination. The increased laccase activity and growth exhibited by *P. ostreatus* in comparison with *C. rivulosus* when inoculated on most forages except *L. perenne* is an indication that different species of fungi vary in their activity, depending on the available substrates ((Patel *et al.*, 2009). The increased activity of CR on LP might be associated with its high nitrogen content. This supports the findings by Hakala *et al.* (2005) where increased laccase activity was recorded when the crude protein extract was concentrated to monitor the production of laccase isoforms grown on spruce wood chips. Therefore, *P. ostreatus* pre-treated forages are expected to produce more WSC than *C. rivulosus* treated ones, except *L. perenne*, and if the filtration method is applied, a greater soluble loss might be recorded.

Influence of filtration methods on the ability of fungi to improve the nutritive content of pre-treated forages

The greater soluble losses in the pre-treated forages using the LFM method as opposed to the FFM method results in no or less positive impact on the fungal ability to improve the chemical composition of the pre-treated forages. This supports the findings of Arora and Sharma (2009b) and Zuo *et al.*, (2018), who identified that WSC are needed to increase the nutritive composition of pre-treated forages and their loss is detrimental to the improvement of the chemical composition of pre-treated forages. The lesser increase of CP content of pre-treated forages obtained from LFM as against that of FFM is an indication that the absolute protein content of the forages might not have changed, but the added fungal biomass (i.e. mycelium/chitin) which was found in both pre-treated forages obtained from both methods might have contributed to the increased CP content recorded. This supports the findings that increased fungal biomass on the substrate is associated with the increased CP content

(Niu *et al.*, 2018; Zuo *et al.*, 2018). The lesser OM reduction in the LFM method as against that of the FFM method was not expected. This can be linked to the fact that the FFM method still retained the WSC as well as the ash content but on the other hand the LFM method removed most of the WSC and ash content, thus increasing the percentage of OM content in the leftover substrates.

The little or no reduction in structural components (i.e. fibre and predicted lignin contents) of the pre-treated forages obtained from the LFM method as against the FFM method indicates that WSC were lost with this method. The obtained results were not in agreement with previous findings where reduction in fibre composition was recorded in fungal pre-treated forages (Niu *et al.*, 2018; Zuo *et al.*, 2018). This further ascertained that loss of WSC through any form of pressure is detrimental to the improvement of pre-treated forages and this must be avoided when handling pre-treated forages. The similar reduction in the secondary metabolite contents and TAC of the pre-treated forages obtained from both LFM and FFM methods is an indication that loss of WSC using any form of filtration method has little effect on metabolite contents of treated forages. In addition, the expectation was that the pre-treated forages obtained from the FFM method would increase the metabolite contents, as lignin degradation took place with minimal soluble loss compared to the LFM method. The degradation of lignin phenolic covalent bonds was expected to be accompanied with the release of more phenolic compounds and total antioxidant contents, as identified in the findings of Lateef *et al.* (2008), Arora *et al.* (2011) and Pouteau *et al.* (2003), however, this was not the case. The difference might be attributed to the lignin molecular weight/ group (Pan *et al.*, 2006), as lignin with less aliphatic, more phenolic hydroxyl groups, wide poly disparity and high molecular weight, does lower the ability of the treated forages to be improved in terms of total phenolic and total antioxidant contents (Pan *et al.*, 2006).

The higher soluble loss recorded by *P. ostreatus* than *C. rivulosus* is an indication that it exhibited higher lignocellulose degradation in most of the pre-treated forages through its increased enzymatic functions, and this is evident in the quantity of soluble contents liberated (Maza *et al.*, 2015). The increased soluble loss is expected to be complemented with an increase in insoluble fractions, i.e. fibre and lignin. However, the lesser reduction in fibre recorded in some of the pre-treated forages from LFM method might be that, as the inoculation time increased, some of the pre-treated forages became porous, therefore reducing the drastic effect that the filtration method might have exerted on the fibre composition. Biological pre-treatment of a substrate has been found to increase the porous nature of pre-treated substrate in readiness for further enzymatic degradation of lignocellulose (Yu *et al.*, 2009). The increased nutrient losses (i.e. fibre, lignin and metabolites) and nutrient gain (CP and ash) in pre-treated forages obtained from the FFM method rather than the LFM method were not expected. The expectation was that the LFM method would give more nutrient loss, since the method has supported more soluble loss. This might be that the leftover substrate no longer reflect the right composition of what might normally be expected if not pump filtered. The obtained results from the FFM method were in agreement with other research findings (Tuyen *et al.*, 2013). Secondary metabolite loss can also be attributed to the fungi utilising this for their growth and activity (Niladevi and Prema, 2008). The obtained results were not in agreement with the findings of Assi and King (2007) and Lateef *et al.* (2008) who recorded gain in the phenolic compounds (i.e. total antioxidant) content of some pre-treated agricultural residues and tomato pomace. This may be because the phenolic compounds constituents were not toxic to the fungi, thus inducing mediators that facilitated the fungal ligninolytic enzymatic potential for increased detoxification of these compounds ((Irshad *et al.*, 2011). The exhibition of more nutrient loss by *P.*

ostreatus than *C. rivulosus* indicates that *P. ostreatus* degrading potential is high (Patel *et al.*, 2009) or that it might have to use the nutrients for its mycelium growth (nee'Nigam *et al.*, 2009). The filtration method has been identified to have influenced the ability the fungi had to improve the chemical composition of the pre-treated forages under the growth conditions selected; the LFM method influenced it negatively while the FFM method influenced it positively.

Conclusions

The use of growth conditions that supports optimal fungal growth and activity might be detrimental to the benefits expected from pre-treated forages if wrong culturing materials or post processing method are not properly carried out. In this study the substrate-liquid ratio of 1:5 and longer inoculation time supported increased growth and laccase activity of the fungi on most of the forages which made more WSC available. These conditions, therefore, helped in achieving more soluble loss and led to no or lesser ability of the fungi to improve the nutritive content of the pre-treated forages based on the processing method i.e. LFM method used as against FFM method. More pronounced detrimental effect was found in *P. ostreatus* treated forages. It is therefore recommended from this study that loss of WSC through any form of pressure, wrong processing or through the use of porous container should be avoided during and after pre-treatment of the substrate as it might be detrimental to the purpose of pre-treatment which is to improve nutritive content of the pre-treated forages. The use of non-porous culturing materials and drying that prevents loss of WSC e.g. freeze drying or drying in containers should be used.

References

- Akinfemi, A. 2010. Nutritive value and in vitro gas production of fungal treated maize cobs, *African Journal of Food, Agriculture, Nutrition and Development*, 10(8).
- A. O. A. C. 1990. Official methods of analysis, *Association of official Analytical Chemist*, 14th Edition, Washington, D. C., p. 2044pp.
- Arora, D. S. and Sharma, R. K. 2009a. Comparative ligninolytic potential of *Phlebia* species and their role in improvement of in vitro digestibility of wheat straw, *Journal of Animal and Feed Sciences*, 18(1), pp. 151-161.
- Arora, D. S. and Sharma, R. K. 2009b. Enhancement in in vitro digestibility of wheat straw obtained from different geographical regions during solid state fermentation by white rot fungi, *BioResources*, 4(3), pp. 909-920.
- Arora, D. S., Sharma, R. K. and Chandra, P. 2011. Biodelignification of wheat straw and its effect on in vitro digestibility and antioxidant properties, *International Biodeterioration and Biodegradation*, 65(2), pp. 352-358.
- Assi, J. A. and King, A. J. 2007. Assessment of selected antioxidants in tomato pomace subsequent to treatment with the edible oyster mushroom, *Pleurotus ostreatus*, under solid-state fermentation, *Journal of Agricultural and Food Chemistry*, 55(22), pp. 9095-9098.
- Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" the FRAP assay, *Analytical Biochemistry*, 239(1), pp. 70-76.
- Daniel, G. 2016. Chapter 8 - Fungal Degradation of Wood Cell Walls A2 - Kim, Yoon Soo, in Funada, R. and Singh, A.P. (eds.) *Secondary Xylem Biology*. Boston: Academic Press, pp. 131-167.
- Geng, X., Li, K. and Xu, F. 2004. Investigation of hydroxamic acids as laccase-mediators for pulp

- bleaching, *Applied Microbiology and Biotechnology*, 64(4), pp. 493-496.
- Goering, H. K. and Van Soest, P. J. 1970.** Forage fiber analyses (apparatus, reagents, procedures, and some applications), *USDA Agr Handb.*
- Hakala, T. K., Lundell, T., Galkin, S., Maijala, P., Kalkkinen, N. and Hatakka, A. 2005.** Manganese peroxidases, laccases and oxalic acid from the selective white-rot fungus *Physisporinus rivulosus* grown on spruce wood chips, *Enzyme and Microbial Technology*, 36(4), pp. 461-468.
- Irshad, M., Asgher, M., Sheikh, M. A. and Nawaz, H. 2011.** Purification and characterization of laccase produced by *Schizophyllum commune* IBL-06 in solid state culture of banana stalks, *BioResources*, 6(3), pp. 2861-2873.
- Lateef, A., Oloke, J. K., Kana, E. B. G., Oyeniyi, S. O., Onifade, O. R., Oyeleye, A. O., Oladosu O. C. and Oyelami, A. O. 2008.** Improving the quality of agro-wastes by solid-state fermentation: enhanced antioxidant activities and nutritional qualities, *World Journal of Microbiology and Biotechnology*, 24(10), pp. 2369-2374.
- Mahesh, M. S. and Mohini, M. 2013.** Biological treatment of crop residues for ruminant feeding: A review, *African Journal of Biotechnology*, 12(27).
- Maza, M., Pajot, H. F., Amoroso, M. J. and Yasem, M. G. 2015.** In-vitro degradation of Czapek and molasses amended post-harvest sugarcane residue by lignocellulolytic fungal strains, *International Biodeterioration and Biodegradation*, 104, pp. 118-122.
- nee'Nigam, P. S., Gupta, N. and Anthwal, A. 2009.** Pre-treatment of agro-industrial residues, *for Agro-Industrial Residues Utilisation*, p. 13.
- Niladevi, K. N. and Prema, P. 2008.** Immobilization of laccase from *Streptomyces psammoticus* and its application in phenol removal using packed bed reactor, *World Journal of Microbiology and Biotechnology*, 24(7), pp. 1215-1222.
- Niu, D., Zuo, S., Jiang, D., Tian, P., Zheng, M. and Xu, C. 2018.** Treatment using white rot fungi changed the chemical composition of wheat straw and enhanced digestion by rumen microbiota in vitro, *Animal Feed Science and Technology*, 237, pp. 46-54.
- Pan, X., Kadla, J. F., Ehara, K., Gilkes, N. and Saddler, J. N. 2006.** Organosolv ethanol lignin from hybrid poplar as a radical scavenger: relationship between lignin structure, extraction conditions, and antioxidant activity, *Journal of Agricultural and Food Chemistry*, 54(16), pp. 5806-5813.
- Patel, H., Gupte, A. and Gupte, S. 2009.** Effect of different culture conditions and inducers on production of laccase by a basidiomycete fungal isolate *Pleurotus ostreatus* HP-1 under solid state fermentation, *BioResources*, 4(1), pp. 268-284.
- Pouteau, C., Dole, P., Cathala, B., Averous, L. and Boquillon, N. 2003.** Antioxidant properties of lignin in polypropylene, *Polymer Degradation and Stability*, 81(1), pp. 9-18.
- Sharma, R. K. and Arora, D. S. 2010.** Production of lignocellulolytic enzymes and enhancement of in vitro digestibility during solid state fermentation of wheat straw by *Phlebia floridensis*, *Bioresource Technology*, 101(23), pp. 9248-9253.
- Sharma, R. K. and Arora, D. S. 2015.** Fungal degradation of

- lignocellulosic residues: An aspect of improved nutritive quality, *Critical Reviews in Microbiology*, 41(1), pp. 52-60.
- Singleton, V. L. and Rossi, J. A. 1965.** Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American journal of Enology and Viticulture*, 16(3), pp. 144-158.
- Tuyen, D. V., Phuong, H. N., Cone, J. W., Baars, J. J. P., Sonnenberg, A. S. M. and Hendriks, W. H. 2013.** Effect of fungal treatments of fibrous agricultural by-products on chemical composition and in vitro rumen fermentation and methane production, *Bioresource Technology*, 129, pp. 256-263.
- van Kuijk, S. J. A., Sonnenberg, A. S. M., Baars, J. J. P., Hendriks, W. H. and Cone, J. W. 2015.** 'Fungal treated lignocellulosic biomass as ruminant feed ingredient: A review', *Biotechnology Advances*, 33(1), pp. 191-202.
- Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991.** Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, *Journal of dairy science*, 74(10), pp. 3583-3597.
- Yu, H., Guo, G., Zhang, X., Yan, K. and Xu, C. 2009.** The effect of biological pretreatment with the selective white-rot fungus *Echinodontium taxodii* on enzymatic hydrolysis of softwoods and hardwoods, *Bioresource Technology*, 100(21), pp. 5170-5175.
- Zuo, S., Niu, D., Zheng, M., Jiang, D., Tian, P., Li, R. and Xu, C. 2018.** Effect of *Irpex lacteus*, *Pleurotus ostreatus* and *Pleurotus cystidiosus* pretreatment of corn stover on its improvement of the in vitro rumen fermentation, *Journal of the Science of Food and Agriculture*.

Received: 4th November, 2019

Accepted: 23rd February, 2020