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Release of Ferulic Acid from Oat Hulls by *Aspergillus* Ferulic Acid Esterase and *Trichoderma* Xylanase

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Oat hulls, an agricultural byproduct, contain a relatively high amount of ferulic acid (FA; 4-hydroxy-3-methoxycinnamic acid), which is believed to be inhibitory to oat hull biodegradability by rumen microorganisms. In this paper, *Aspergillus* ferulic acid esterase (FAE) was investigated for its ability to release FA from oat hulls. The objectives were to determine the effects of particle size of oat hulls (ground to pass through 1 mm and 250 μm screens and a 100 μm sieve) on release of FA by FAE both in the presence and in the absence of *Trichoderma* xylanase. The results show that the release of FA by FAE was dependent upon the particle size of oat hulls ($\leq$250 μm). In the absence of *Trichoderma* xylanase, little FA was released by FAE. In the presence of *Trichoderma* xylanase, there was a significant release of FA by FAE, indicating a synergistic interaction between FAE and *Trichoderma* xylanase on release of FA from oat hulls. These results indicate that FAE is able to break the ester linkage between FA and the attached sugar, releasing FA from oat hulls. This may leave the remainder of the polysaccharides open for further hydrolytic attack by rumen microorganisms. It is likely that removing FA from oat hulls could improve rumen biodegradability, thus improving the nutritional value of oat hulls.

KEYWORDS: ***Aspergillus* ferulic acid esterase; ferulic acid; hydroxycinnamic acid; phenolic acids; oat hulls; complex cell walls; lignocellulose; biodegradation**

INTRODUCTION

Oat hulls are a byproduct of the oat-processing industry. In western Canada, ~350000 metric tonnes of oats is processed annually. The hull represents 26−31% of the weight of the oat seed (*1*). This leaves in excess of 100000 metric tones of oat hulls available to the feed industry in western Canada (V. J. Racz, 2000, unpublished observation). Nutritionally, oat hulls are high in fiber, low in protein, and comparable to cereal straw as a feedstuff in terms of ruminant nutritional values such as energy and available crude protein (*1*). As such, they are suitable only for ruminant feed. Due to the large supply, it is economically important to improve the nutritional qualities of this byproduct.

A great deal of research effort has been expended on the identification and examination of factors affecting fiber fermentation by ruminants. This reseach has focused on factors such as core lignin composition and concentration, lignin−carbohydrate and phenolic−carbohydrate complexes, lignin encrustation, hemicellulose encrustation, and cellulose crystallinity (*2*−*6*) as reviewed by Garleb et al. (*7*). Garleb et al. (*7*) stated that lignin encrustation and the presence of lignin−carbohydate/phenolic−carbohydrate complexes are two important factors that inhibit the digestibility of oat hulls by rumen microorganisms. Graham and Åman (*8*) and Hartley and Ford (*9*) stated that hindrance of lignocellulose biodegradation is associated with phenolic components. The phenolic acids are produced via the phenylpropanoid biosynthetic pathway (*10*, *11*). They may act as a cross-linking agent between lignin and carbohydrates or between carbohydrates (*10*, *12*−*17*). Various studies have consistently indicated that phenolic constituents are among the factors most inhibitory to the biodegradability of plant cell wall polysaccharides (*18*−*24*).

Ferulic acid (FA; 4-hydroxy-3-methoxycinnamic acid) is the most abundant hydroxycinnamic acid in the cell walls of cereal grains (*15*, *16*, *25*, *26*), and it is also a major phenolic acid found in oat hulls (*6*, *7*, *13*, *27*). It is covalently cross-linked to polysaccharides by ester bonds and to components of lignin mainly by ether bonds (*13*, *15*, *22*, *28*, *29*). It is also possible to dimerize between esterified and (or) etherified FA residues by oxidative coupling (*8*, *15*, *16*, *24*, *26*, *30*, *31*). These cross-links have a number of physical consequences: they negatively affect nutritional value, increase the mechanical strength of the cell wall, and limit cell wall growth (*9*, *32*). These cross-links not only contribute to the control of cell wall extensibility and growth (*8*) but also significantly limit cell wall degradability

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by rumen microorganisms (*14*, *32*, *33*) and thus limit digestibility by ruminants (*15*, *23*, *24*).

Recently a novel enzyme, ferulic acid esterase (FAE), was found to break the ester linkage between FA and the attached sugar and release FA from complex cell walls, such as wheat bran (*14*, *16*, *34*), sugar beet pulp (*25*), and barley spent grain (*26*). FAE has been purified and characterized from *Aspergillus niger* (*10*, *15*, *25*, *35*). The proportion of FA released from wheat bran and barley spent grain by FAE further increases in the presence of xylanase (*14*, *26*, *34*). Efficient release of FA by FAE has been shown to be affected by substrate particle size (1 mm vs 80 μm) as reported by Borneman et al. (*22*).

To this point, no work has been carried out on the enzymic release of FA from oat hulls by FAE. The objectives of this study were to investigate the ability of *Aspergillus* FAE to release FA from oat hulls and determine the effects of particle size of oat hulls on enzymic release of FA by the action of *Aspergillus* FAE, both in the presence and in the absence of *Trichoderma* xylanase.

MATERIALS AND METHODS

Oat Hulls and Particle Size. Oat hulls were obtained from Can-Oat Milling Ltd. at Martinsville, SK, and used throughout the experiment. Oat hulls were first screened to remove grain, small kernels, and other foreign materials and then ground through 1 mm and 250 μm screens. Further processing involved using a 100 μm size USA Standard Testing Sieve to obtain a finer particle size.

Chemical Analysis. Oat hulls were analyzed for moisture, ash, acid detergent fiber, lignin, and crude protein according to the procedures of the Association of the Official Analytical Chemists (*36*). Neutral detergent fiber was determined according to the procedure of Van Soest et al. (*37*) without sodium sulfite.

Enzymes and Assays. *Aspergillus* FAE (lot 99021904) and *Trichoderma* xylanase (lot 990215-04) were obtained from Finnfeeds International. The FAE activity was determined by measuring the rate of hydrolysis of methyl ferulate (MF; methyl-4-hydroxy-3-methoxy cinnamate) (Apin Chemicals Limited) using the modified method from Faulds and Williamson (*14*) and Kroon and Williamson (*25*). One unit (U) of FAE activity was defined as the amount of enzyme releasing 1 μmol of FA/min at pH 6.0 at 37 °C. *Trichoderma* xylanase activity was estimated by measuring the release of reducing sugars from soluble oat spelt xylan (Sigma, X-0627, lot 70H094) (*38*) and expressed as xylose equivalents [Sigma-D (+), X-3877] (*39*). One unit of activity was defined as the amount of enzyme releasing 1 μmol of sugar/min at pH 4.8 at 37 °C. All assays were performed in triplicate, with blanks to correct for background in enzyme and substrate samples. The activities of *Aspergillus* FAE and *Trichoderma* xylanase were 46784 (SD = 368; $n = 3$) U mL^{-1} and 579369 (SD = 11528; $n = 3$) U g^{-1}, respectively.

Chemical Hydrolysis. Total alkali-extractable FA content of oat hulls (10 mg) was determined by adding 1 M NaOH solution (0.55 mL) followed by incubation at 37 °C for 24 h. After centrifugation (13000*g*, 15 min), the supernatant was collected, acidified with glacial acetic acid to pH 3, and extracted five times with equal volumes of ethyl acetate. The organic solutions were combined and evaporated to dryness in an evaporator unit under N_2. The residue was dissolved in 1 mL of methanol/water (50:50, v/v) filtered through a 0.45 μm filter, and 10 μL samples were analyzed by HPLC (*25*). Samples were prepared and analyzed in triplicate.

Enzymic Hydrolyses. Enzymic hydrolyses were carried out in 0.1 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer, pH 6.0, in a thermostatically controlled shaking incubator at 37 °C. Oat hulls (10 mg) were incubated with different amounts of FAE and (or) *Trichoderma* xylanase in a final volume of 0.55 mL. The enzyme concentrations used for assay are presented in **Tables 1** and **2**.

After incubation, the reaction was stopped by adding glacial acetic acid. The samples were centrifuged (13000*g*, 15 min), the supernatant was extracted into ethyl acetate five times and pooled, dried samples were taken up in 50% methanol and filtered through a 0.45 μm filter, and 10 μL samples were analyzed by HPLC (*25*). Each sample was prepared and analyzed in triplicate.

Table 1. Release of Ferulic Acid from Oat Hulls by Different Concentrations of the *Aspergillus* Ferulic Acid Esterase (FAE 0–3276.8 U/Assay) after a 24 h Incubation in the Absence of the *Trichoderma* Xylanase[a]

	release of FA from oat hulls (% of total FA)	
FAE (mU or U/assay)	250 μm	100 μm
0 (control)	0.31 b	0.58 e
3.1 mU/assay	0.08 b	0.74 e
6.25 mU/assay	0.40 b	1.04 de
12.5 mU/assay	0.77 b	1.04 de
25 mU/assay	0.54 b	0.54 e
50 mU/assay	0.49 b	1.05 de
90 mU/assay	0.39 b	0.49 e
130 mU/assay	0.45 b	0.64 e
260 mU/assay	0.79 b	1.00 e
400 mU/assay	0.85 b	0.84 e
800 mU/assay	1.38 b	1.32 cde
1.6 U/assay	1.11 b	1.47 cde
3.2 U/assay	1.14 b	1.57 cde
6.4 U/assay	1.04 b	1.55 cde
12.8 U/assay	0.44 b	2.13 bcde
26 U/assay	1.36 b	2.70 bc
51.2 U/assay	1.47 b	1.79 cde
102.4 U/assay	1.30 b	2.51 bcd
204.8 U/assay	1.86 b	3.20 b
409.6 U/assay	2.83 b	3.38 b
819.2 U/assay	3.19 ab	5.97 a
1638.9 U/assay	4.17 a	6.28 a
3276.9 U/assay	4.66 a	6.92 a
SEM[b]	0.542	0.319

[a] 100% FA is defined as the content of total alkali-extractable ferulic acid in oat hulls. [b] SEM, standard error of mean; means with different letters in the same row are significantly different ($P < 0.05$). Each mean is based on triplicate measurements.

Table 2. Influence of *Trichoderma* Xylanase Concentration (0–32768 U/Assay) on Release of Ferulic Acid from Oat Hulls by *Aspergillus* Ferulic Acid Esterase (26 U/Assay) after a 24 h Incubation[a]

		release of FA from oat hulls (% of total FA)	
xylanase (mU or U/assay)	FAE (U/assay)	250 μm	100 μm
0 (control)	26 U/assay	0.66 j	0.63 j
7.8 mU/assay	26 U/assay	1.86 j	0.65 j
15.6 mU/assay	26 U/assay	1.33 j	2.04 j
31.3 mU/assay	26 U/assay	0.74 j	2.52 j
62.5 mU/assay	26 U/assay	0.82 j	1.13 j
125 mU/assay	26 U/assay	0.83 j	0.74 j
250 mU/assay	26 U/assay	1.74 j	1.02 j
500 mU/assay	26 U/assay	2.73 j	1.88 j
1 U/assay	26 U/assay	4.35 ij	1.07 j
2 U/assay	26 U/assay	3.54 j	2.18 j
4 U/assay	26 U/assay	4.38 ij	2.62 j
8 U/assay	26 U/assay	4.22 ij	7.13 i
16 U/assay	26 U/assay	3.69 j	10.55 h
32 U/assay	26 U/assay	4.55 ij	10.90 h
64 U/assay	26 U/assay	7.45 i	12.29 gh
128 U/assay	26 U/assay	11.80 h	14.91 g
256 U/assay	26 U/assay	17.42 g	14.56 g
512 U/assay	26 U/assay	18.76 g	21.14 f
1,024 U/assay	26 U/assay	22.15 f	25.29 e
2,048 U/assay	26 U/assay	27.16 e	26.89 e
4,096 U/assay	26 U/assay	33.34 d	33.80 d
8,192 U/assay	26 U/assay	43.04 c	38.43 c
16,348 U/assay	26 U/assay	52.35 b	50.12 b
32,768 U/assay	26 U/assay	68.52 a	62.54 a
SEM[b]		0.864	1.010

HPLC Conditions. For HPLC [a Beckman chromatograph equipped with a 126 programmable solvent module, a 507 autosampler (System Gold), and an RF-551 PC spectrofluorometric detector], a mobile phase with 3.1% methanol, pH 9.5, and 20 mM K_2HPO_4 was applied to a PRP-1 column (Polymerx, PRP-1, 150 × 4.6 mm, 5 μm, pH 1−13, Phenomenex). The flow rate was 1 mL min^{-1}, with detection at 280 nm excitation and 428 nm emission (response time, 1.5 s; range, ×4; sensitivity, high), and the temperature of the column was maintained at room temperature (~25 °C).

Ferulic acid in samples was quantified using external standards of FA (Sigma, F-3500, lot 48H0416, formula weight = 194.2). Standard solutions (between 2 and 100 μmol) were subjected to the ethyl acetate extraction procedure described above. Calibration curves were calculated on the basis of the linear correlation between concentration of standards and the height of the FA peak.

Statistical Analysis. Statistical analyses on effects of FAE alone or xylanase levels in the presence of 26 U/assay of FAE on FA release from oat hulls were carried out as a completely randomized design using the GLM procedure of SAS (*40*) with the model

$$Y_{ik} = \mu + E_i + e_{ik} \quad (1)$$

where Y is the dependent variable under examination (% FA), μ the overall mean, E_i the FAE effect or xylanase effect, and e_{ik} the error term.

Treatment means were compared using the Student−Newman−Keuls (SNK) test (*41*). Significance was declared at $P < 0.05$.

Linear and quadratic effects of FAE and (or) xylanase levels on FA release from oat hulls were also evaluated by polynomial regression analysis with the GLM procedure of SAS (*40*), using the following additive model. Before tests of significance in polynomial regression analysis, the UNIVARIATE procedure of SAS was used to test residuals for normality.

$$Y_i = \alpha + \beta_1 X + \beta_2 X^2 + e \quad (2)$$

In eq 2, Y_i is the dependent variable (FA) under examination, α the intercept, X the FAE or xylanase effect (in U/assay), β_1 the linear effect of FAE or xylanase, β_2 the quadratic effect of FAE or xylanase, and e the residual error term.

RESULTS

Total Content of Ferulic Acid and Chemical Composition of Oat Hulls. Total alkali-extractable FA in oat hulls used in this study was 3.83 μg mg^{-1} dry matter (DM) (or 4.94 μg mg^{-1} neutral detergent fiber). Oat hulls contain 44 g kg^{-1} DM of crude protein, 52 g kg^{-1} DM of ash, 775 g kg^{-1} DM of neutral detergent fiber, 405 g kg^{-1} DM of acid detergent fiber, and 56 g kg^{-1} DM of lignin.

Effects of Particle Size of Oat Hulls on Release of Ferulic Acid by *Aspergillus* Ferulic Acid Esterase. No release of FA was detected following the incubation of oat hulls ground through a 1 mm screen for 24 h with FAE alone. Release of FA from oat hulls ground through a 250 μm screen and a 100 μm sieve by FAE at different concentrations (0−3276.9 U/assay) after a 24 h incubation is given in **Table 1**. With increasing FAE level, the release of FA was slightly increased from oat hulls ground through a 250 μm screen. Reducing particle size toward very finely ground (100 μm) oat hulls did not significantly increase ($P > 0.05$) FA release. The highest releases of FA were 4.7% from oat hulls ground through a 250 μm screen and 6.9% from oat hulls ground through a 100 μm screen at a FAE concentration of 3276.9 U/assay. Polynomial regression analysis indicated a quadratic effect ($P < 0.001$) of FAE concentration on FA release from oat hulls with particle sizes of both 250 and 100 μm.

Effects of *Trichoderma* Xylanase on Release of Ferulic Acid from Oat Hulls by *Aspergillus* Ferulic Acid Esterase. Influence of the *Trichodema* xylanase concentration (0−32768 U/assay) on the release of FA from oat hulls by FAE (26 U/assay) after a 24 h incubation is presented in **Table 2**. The concentration of FAE of 26 U/assay was chosen in this trial due to its intermediate release of FA from oat hulls ground through a 250 μm screen and a 100 μm sieve by FAE alone. As *Trichoderma* xylanase concentration increased, the release of FA by FAE (at 26 U/assay) was greatly increased from 0.7 to 68.5% from oat hulls ground through a 250 μm screen and from 0.6 to 62.5% from oat hulls ground through a 100 μm sieve. Polynomial regression indicated that FA release increased quadratically ($P < 0.001$) as xylanase concentration increased when FAE level was held constant (26 U/assay).

DISCUSSION

Total Ferulic Acid Content of Oat Hulls. The FA content of oat hulls used in this study (expressed as either μg mg^{-1} DM or μg mg^{-1} neutral detergent fiber) was slightly higher than two other published results: 2.36 μg mg^{-1} DM (or 3.12 μg mg^{-1} neutral detergent fiber) (*7*) and 2.15 μg mg^{-1} DM (or 2.99 μg mg^{-1} neutral detergent fiber) (*6*). These differences again can be due to the varietal difference of oat hulls used in the different studies (*42*).

Effect of Type of *Aspergillus* Ferulic Acid Esterase and Substrate on Release of Ferulic Acid. Studies show that the type of FAE affects the release of FA. The FAE from *Streptomyces olivochromogenes* (*10*) and FAE I from *Aspergillus niger* (*35*) alone could not release FA from wheat bran. However, FAE II and III from *Aspergillus niger* alone were shown to release FA from wheat bran (*14*, *35*) and barley spent grains (*26*). The type of not only the FAE but also the substrate will affect the release of FA. Brezillon et al. (*15*) reported that FAE III from *Aspergillus niger* alone could not release FA from sugar beet pulp (dicot) but released FA from wheat bran (monocot). Our study showed that *Aspergillus* FAE alone could release a small amount of FA from oat hulls that were ground to pass through a 250 μm screen and a 100 μm sieve.

Effect of Substrate Particle Size on Activity of *Aspergillus* Ferulic Acid Esterase. The present study showed that release of FA was affected by particle size of oat hulls. The FAE could not release FA from oat hulls ground through a 1 mm screen; however, when particle size was reduced (250 and 100 μm), a small release of FA occurred. This may be due to the fact that the larger particle size of the substrate of oat hulls may restrict accessibility of the esterase to the feruloyl groups and thus impede enzyme activity, resulting in no detectable release of free FA from oat hulls. Borneman et al. (*22*) also found that the purified FAE had no detectable activity toward coastal bermuda grass cell walls that were ground to pass through a 1 mm sieve but had low levels of activity toward very finely ground (80 μm) cell walls. Both experiments clearly indicate that the activity of FAE is affected by substrate particle size.

Effect of Specificity of *Aspergillus* Ferulic Acid Esterase on Release of Phenolic Acids. Faulds and Williamson (*43*) found that FAE from *A. niger* could release FA and sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) but not *p*-coumaric acid (4-hydroxycinnamic acid) or caffeic acid (3,4-dihydroxycinnamic acid). This indicates that FAE is active only on methyl esters of certain hydroxycinnamic acids, including ferulic and sinapic esters, but not *p*-coumaric or caffeic esters (*43*). This also shows that only certain substitutions on the aromatic ring can be tolerated, and this gives specificity to this enzyme. In contrast, FAE from *Pseudomonas fluorescens* ssp. *cellulosa* is effective on all of these methyl hydroxycinnamic esters (*43*).

Ralet et al. (*23*) reported that FAE I from *A. niger* shows no clear preference for the type of linkage involved between the FA units and the oligosaccharide chains, but FAE III from *A. niger* has a clear requirement for FA to be attached to O-5 of the arabinofuranose ring, whereas no catalysis was observed when FA was attached to O-2. These findings indicate that FAE I from *A. niger* can cause the release of FA from both monocots (such as wheat, oats, and barley) and dicots (sugar beet), but FAE III from *A. niger* can release FA only from monocots. This is because in monocots, FA is esterified to the C-5 hydroxyl group of some arabinopyranose residues of arabinoxylans (*21*, *24*, *44*), but in dicots, FA is esterified to the C2-hydroxyl group of arabinofuranose or to the C-6 hydroxyl group of galactopyranose residues of the pectic side chains as reported by Hartley et al. (*21*), Ralet et al. (*23*), Kroon et al. (*24*), and Bartolomé and Gòmez-Cordovés (*44*).

Synergistic Interaction between *Aspergillus* Ferulic Acid Esterase and *Trichoderma* Xylanase. The results of this study indicate that release of FA from oat hulls by FAE was increased after the addition of *Trichoderma* xylanase. With increasing level of *Trichoderma* xylanase, FA release was further increased. For example, FAE alone at 26 U/assay released only 1.4% of total FA from oat hulls (250 μm), but after the addtion of *Trichoderma* xylanase, the release of FA was increased to 69%. These results clearly indicate a synergistic interaction between FAE and *Trichoderma* xylanase. *Trichoderma* xylanase strongly influences the action of *Aspergillus* FAE on oat hulls. This synergistic interaction might be related to the rate of hydrolysis of oat hulls by the two enzymes and the specificity of the xylanase to release fragments more suitable for attack by the esterase.

Our results are in agreement with the findings of MacKenzie and Bilous (*45*) and Faulds and Williamson (*10*), in that the FAE (from *Streptomyces olivochromogenes* and *Schizophyllum commune*) was unable to release FA from wheat bran in the absence of xylanase, but it could interact synergistically with the xylanase to release FA. Faulds and Williamson (*14*) also reported that FA was efficiently released from a wheat bran preparation by a FAE from *A. niger* (FAE) when incubated together with a *Trichoderma viride* xylanase (a maximum of 95% total FA released after a 5 h incubation). *Aspergillus* FAE by itself could release FA but at a level almost 24-fold lower than that obtained in the presence of xylanase. Bartoloma et al. (*16*, *26*) concluded that FAE released free FA from cell walls only in the presence of a xylanase.

On the basis of these results, it can be hypothesized that in order to efficiently release free FA from complex plant cell walls, a two-step process occurs. Specific enzymes such as xylanase are first required to solubilize part of the cell wall structure by formation of low relative molecular weight feruloylated compounds. Then FAE can act on these feruloylated compounds to release FA (*14*, *34*, *46*). The initial enzymatic hydrolysis is expected to alter the physical properties of the cell wall and make it more accessible to further enzymatic attack. The initial enzymic attack alters also the chemical properities of the cell wall, as cleavage of the linear xylan backbone yields a number of shorter oligo- and (or) polymers. However, the mode of action and hydrolysis products may vary according to the specific accessary cell wall degrading enzyme and the source of the enzyme.

Effect of Complex Cell Wall Materials on Release of Ferulic Acid. The present study showed that *Aspergillus* FAE could release 69% (highest amounts) of FA from oat hulls in the presence of *Trichoderma* xylanase. This result was lower than that from wheat bran (95%) (*14*, *47*) but higher than that from barley spent grain (30%) (*26*).

These studies indicate that enzymic release of FA by FAE may be affected by the complexities of cell wall materials (such as lignification). Wheat bran contains essentially nonlignified cell walls and is therefore more susceptible to enzymic degradation, but oat hulls are very lignified and are therefore less susceptible to the esterase (*42*).

Reasons for the discrepancy in release of FA may also be due to physical and steric factors, presumably by the branching of the respective arabinoxylans. Bartolomé et al. (*26*) reported that FAE was able to release 30% of FA from barley spent grain. The structure of arabinoxylans in barley spent grain consists of β-(1→4) xylans, in which xylose residues are substituted with arabinose at C2 (17% of the total xylose residues), C3 (7%), or both C2 and C3 (16%). Feruloyl groups are 5-O attached to arabinofuranosyl residues (*26*, *48*, *49*). Fauld et al. (*47*) found that FAE was able to release up to 95% of FA from wheat bran. The structure of arabinoxylans in wheat bran constitutes fewer branched β-(1→4) xylopyranose backbones, with xylose residues substituted mainly at C3 (16% of the total xylose residues) and at both C2 and C3 (4%) (*26*, *50*). Feruloy groups are 5-O attached to arabinofuranosyl residues (*23*, *26*). In contrast, Faulds et al. (*51*) found that even though FAE exhibited significant activities on feruloylated oligosaccharides derived from maize bran, it was unable to release FA from maize bran in the presence of xylanase from *T. viride*. The structure of arabinoxylans from maize bran shows the most-branched structure with 40% single-substituted xylose (C2 or C3) and 20% double-substituted xylose (C2 and C3) (*52*). Arabinose residues are found in terminal positions (60%) and in short side chains (40%). Feruloyl groups are again 5-O attached to arabinofuranosyl residues (*26*, *53*). It appears, therefore, that less-substituted xylan substrates such as wheat bran and barley spent grain are better substrates for FAE release of FA than more substituted substrates such as maize bran (*26*). Although the arabinoxylan structure of oat hulls is not known, a high degree of substituents may restrict accessibility of the esterase to the feruloyl groups in the soluble substrate and may explain the lower release of FA with FAE with oat hulls.

Conclusion. In summary, the particle size of oat hulls (ground through 1 mm and 250 μm screens and a 100 μm sieve) affected release of free FA by *Aspergillus* FAE. In the absence of *Trichoderma* xylanase, little release of FA occurred by FAE, no matter how much FAE was added. Especially *Aspergillus* FAE had no effect on FA release from oat hull ground through a 1 mm screen. Reducing particle size (using a 250 μm screen) resulted in only a small amount of FA (highest = 4.7%) released even at the largest concentration of FAE (3276.9 U/assay). No further improvement was observed when particle size was further reduced toward very finely ground (100 μm) oat hulls.

In the presence of *Trichoderma* xylanase, there was a significant release of FA by FAE, indicating a synergistical interaction between *Aspergillus* FAE and *Trichoderma* xylanase on release of FA from oat hulls. With increasing level of *Trichoderma* xylanase, release of FA was increased, with highest release of FA (69 and 63% from oat hulls ground through a 250 μm screen and a 100 μm sieve, respectively) at a *Trichoderma* xylanase concentration of 32768 U/assay.

Implications. Using *Aspergillus* FAE in combination with other cell walls degrading enzymes, *Trichoderma* xylanase could improve rumen biodegradability of poorly digestible complex plant cell walls containing relatively high contents of ferulic acid.

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