Presence of Acetamide in Milk and Beef from Cattle Consuming AFEX-Treated Crop Residues

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Supporting Information

ABSTRACT: AFEX treatment of crop residues can greatly increase their nutrient availability for ruminants. This study investigated the concentration of acetamide, an ammoniation byproduct, in AFEX-treated crop residues and in milk and meat from ruminants fed these residues. Acetamide concentrations in four AFEX-treated cereal crop residues were comparable and reproducible (4−7 mg/g dry matter). A transient acetamide peak in milk was detected following introduction of AFEX-treated residues to the diet, but an alternative regimen showed the peak can be effectively mitigated. Milk acetamide concentration following this transition was 6 and 10 ppm for cattle and buffalo, respectively, but also decreased over time for cattle while tending to decrease (p = 0.08) for buffalo. There was no difference in acetamide concentration in the meat of cattle consuming AFEX-treated residues for 160 days compared to controls. Further investigation is necessary to determine the metabolism of acetamide in ruminants and a maximum acceptable daily intake for humans.

KEYWORDS: acetamide, contaminant, AFEX, milk, food safety

INTRODUCTION

The total demand for animal food products is expected by FAO to more than double by 2030, driven by the growing middle-class population in developing countries.¹ The livestock sector is already the single largest user of the world’s land, accounting for approximately 30% through grazing and growing fodder and feeds.² Increasing livestock productivity will remain a significant challenge for sustainable agriculture.

One solution to increase ruminant livestock productivity, without increasing land use, is to improve the digestibility of cereal crop residues such as rice straw, wheat straw, and corn stover that are produced wherever grains are grown. In South and Southeast Asia in particular, increased usage of upgraded crop residues for cattle feed could simultaneously reduce air pollution caused by crop residues currently being burned on the fields.²,³

Ammoniation of crop residues has been well documented to increase their digestibility, although no widespread implementation of ammoniation technologies has occurred.⁴ One approach to ammoniation is ammonia fiber expansion (AFEX), in which crop residues would be treated with anhydrous ammonia at elevated temperatures and pressures (approximately 100 °C and 2 MPa) for a short residence time (30 min) in packed bed reactors with the ammonia being recovered and recycled at regional collection and processing centers.⁵ Ammoniation makes the cellulose sugar polymers in crop residues more accessible to hydrolytic enzymes and also increases the inorganic nitrogen content, which result in increased digestibility of the crop residues in vitro⁶−⁸ and in vivo.⁹

During the AFEX treatment, a small portion of ammonia reacts with acetate esters in the plant cell wall to form acetamide (CAS no. 60-3-5), which could be considered a feed contaminant. Acetamide has been classified as a possible human carcinogen; rats fed acetamide at 2.36% of their diet for 12 months produced liver carcinomas.¹⁰ While acetamide can be metabolized in the rumen by amidase enzymes into acetate and ammonium,¹¹−¹³ any acetamide that is not metabolized in the rumen could enter the milk or meat, in which case the feed contaminant would also become a food contaminant. Previous research suggests that acetamide can pass through the rumen wall¹⁴ and thus may enter the bloodstream. Recently, it has been shown that acetamide is already present as a food contaminant in many foods, including milk, beef, roasted coffee,¹⁵ and chicory root.¹⁶

While there is potential for AFEX and other ammoniation treatments to upgrade the value of straw, it is important to understand the extent to which feed contaminants such as acetamide could enter the food chain and the extent to which the resulting levels pose a risk to human health. The purpose of this study is to investigate the levels of acetamide formed during AFEX processing and the appearance of acetamide in...
Table 1. Summary of Feeding Trials

| trial no. | type        | animals | animals per treatment | AFEX pellet inclusion | control            |
|-----------|-------------|---------|-----------------------|----------------------|--------------------|
| 1         | dairy       | Murrah buffalo | 10 | low: 25% wheat straw, high: 50% wheat straw | high grain diet prior to AFEX inclusion |
| 2         | dairy       | Karan–Fries cattle | 10 | low: 25% wheat straw, high: 50% wheat straw | high grain diet prior to AFEX inclusion |
| 3         | dairy       | Karan–Fries cattle | 6   | 3% or 10% wheat straw for 1 week, 30% for 1 week | 0% for 1 week, 30% for 1 week |
| 4         | beef        | Holstein steer  | 12   | 30% corn stover | high grain diet |
| 5         | beef        | Holstein calf   | 8    | 50% rice straw | high rice straw diet |

the milk and tissues of animals consuming AFEX-treated crop residues in their diet.

**MATERIALS AND METHODS**

**Materials.** Conventional multipass, low-cob corn stover was harvested in Hamilton County, IA. Wheat straw was harvested in Leslie, MI. Rice straw was harvested from Craighead County, AR, and Butler County, MO. All materials were initially stored in large square bales before being chopped to ∼2.5 cm particle size using a Vermeer BG 480 grinder. Chopped biomass was stored in plastic-lined supersacks at <10% moisture prior to use. AFEX treatment in a packed bed reactor was performed using the method described by Sarks et al. To determine if acetamide accumulates in the meat of cattle, a beef calves trial (Trial 5), beginning in December 2018 in East Lansing, MI, was performed with 16 Holstein calves (150 kg initial body weight) randomly divided into either an AFEX treatment group or the control group. Animals were individually fed an ad-libitum diet for 160 days, followed by a 24 h fasting period and then slaughtered. Diets were 30% AFEX corn stover pellets, 36% corn grain, 10% corn silage, 20% dry distillers grains and solubles (DDGS), and 4% mineral supplement for the AFEX treatment and 51% corn grain, 15% corn silage, 30% DDGS, and 4% mineral supplement for the controls. Samples of loin were collected from each carcass for acetamide analysis.

To confirm the results of Trial 4, a beef calf trial (Trial 5), in brief, milk samples were spiked with propionamide (0.50 mg/mL milk) to use as an internal standard. Samples were then defatted via centrifugation and proteins removed by adding an equal volume of 0.5 M HCl. Acetamide was derivatized by adding 200 μL of 5% 9-xanthydrol solution in methanol to 5 mL of sample and incubated at 40 °C for 1 h. The derivatization was required to prevent interference by acetamide formed from the breakdown of N-acetylated sugars such as N-acetylated glucosamine and sialic acids. The derivatized materials were resolubilized in ethyl acetate and analyzed using GC/MS. Samples of feed were analyzed for acetamide according to the method reported by Chundawat et al. Briefly, acetamide was extracted using water as a solvent in an ASE 200 Accelerated Solvent Extractor (Dionex) at approximately 11 mL of water per gram sample for 2 cycles. The extracts were combined and analyzed using GC-MS.
Results and Discussion

Acetamide Production during AFEX Treatment. A comparison of acetamide produced from different crop residues and different methods of AFEX treatment is shown in Table 2. Acetamide in the pilot-scale packed bed-treated materials ranged from 4 to 7 mg/g dry matter across the four cereal crop residues tested, with an average standard deviation of 0.6–0.9 mg/g within the crop type. Approximately 18–23% of the total acetate in the crop residue was converted to acetamide. Acetamide production was observed to be lower in the pilot-scale packed bed reactor than the laboratory Parr reactor for every crop residue tested. Aside from AFEX pellets, no acetamide was detected in any of the other feed ingredients (data not shown).

Acetamide is produced during AFEX treatment from the reaction of acetyl linkages within the plant cell wall with ammonia. Thus, the amount of acetamide produced during AFEX treatment is limited by two factors: the acetate content in the crop residue (because ammonia is added in excess) and the process conditions (which impact the extent of ammoniation). Acetate content in the stalks and leaves of grasses (including cereal crops) range from 2% to 3% of the total dry matter, consistent with the values observed in this study. The process conditions in the packed-bed reactor were very similar across the four types of crop residues tested, and the variance in acetamide content across different samples of AFEX-treated crop residues was correspondingly low. The packed-bed reactor process is controlled via pressure, which essentially limits the temperature and ammonia concentration, thereby allowing for a practical control on the amount of acetamide produced during the AFEX process.

In contrast, the process conditions in the Parr reactor resulted in the conversion of 29–37% of the acetate into acetamide. The higher conversion relative to the packed bed reactor is likely due to the presence of external heating in the Parr reactor, resulting in localized higher temperatures for biomass near the walls of the reactor. Note that the packed-bed reactor does not have external heating. Chundawat et al. reported a higher value for acetamide in corn stover that was treated using AFEX in a Parr reactor (25 mg/g) compared to the values reported here, and the difference between the two reactors is consistent with findings for sugar cane bagasse (11.1 mg/g vs 15.5 mg/g). The Parr reactor is not relevant for commercial scale-up, and thus, the acetamide formed in the packed-bed reactor is more relevant as a predictor of what may be anticipated in commercial operations.

Although this study is specific to AFEX treatment, it is likely that some acetamide is also produced when other modes of ammoniation are practiced for crop-residue treatment. For example, Van Soest summarizes reports in which an increased nitrogen content is observed in crop residues following ammoniation, though the presence of acetamide per se has not been investigated or quantified. It is likely that AFEX treatment, which constitutes a more complete form of ammoniation, results in higher acetamide content than other traditional ammoniation practices.

Table 2. Acetate Content and Resulting Acetamide Production after AFEX Treatment

| Crop Residue | Acetate Content (mg/g) | Acetamide Content (mg/g) | Acetamide Content in % (dry matter) |
|--------------|------------------------|--------------------------|------------------------------------|
| Corn stover  | 6.6 ± 0.6              | 8.3                      | 2.9%                               |
| Wheat straw  | 5.6 ± 0.7              | 8.6                      | 2.3%                               |
| Rice straw   | 4.4 ± 0.9              | not tested               | 2.0%                               |
| Barley straw | 4.3                    | 8.4                      |                                    |

"Mean and standard deviation is from 20 different batches for corn stover, wheat straw, and rice straw."
metabolize acetamide into acetate and ammonia, suggesting that acetamide can be utilized by ruminants as a nitrogen source. It is likely that acetamidase production is being increased in the rumen during this transient adaptation period, either by a change in subpopulations in the rumen microflora or by a change in acetamidase expression levels. The initial adaptation period occurs over a period of 3−11 days and varies greatly from animal to animal, likely due to differences in their rumen ecosystems and responses.

Altering the manner in which AFEX pellets were first introduced to animals clearly mitigated the transient acetamide peaks, as seen in Figure 2. In Trial 3, crossbred cattle were provided with a fixed ratio of 0%, 3%, or 10% of AFEX pellets in their diet for the first week, followed by a stepwise increase to 30% AFEX pellets inclusion in the second week.

Figure 1. Acetamide concentration in milk over time for both Murrah buffalo (A and B) and Karan−Fries cows (C and D) immediately after introduction of AFEX pellets to the diet. Each line represents a different animal (10 animals total per treatment). Percentage of AFEX pellets in the diet on each day is represented by the dashed line.

Figure 2. Acetamide concentration in milk for individual Karan−Fries cattle from Trial 3 during the transition of diets to AFEX pellets. For the first 7 days shown, animals were given a transition diet of (A) 3% AFEX pellets, (B) 10% AFEX pellets, or (C) the control of 0% AFEX pellets. Animals were provided with 30% AFEX pellets during the next 7 days. Each line represents a different animal, with 6 animals per treatment (5 animals for the control).
mean for 3 (cattle) or 4 (buffalo) species and breeds, these findings are indicative that a feeding regimen can be developed to mitigate any peaks in milk acetamide levels when AFEX pellets are present in the AFEX pellets in the feed.

Table 3. Comparison of Acetamide Concentration to Milk Yield and Intake of AFEX pellets

| Murrah buffalo (Trial 1) | Karan–Fries cattle (Trial 2) |
|--------------------------|-----------------------------|
| low AFEX                 | high AFEX                   |
| low AFEX                 | high AFEX                   |
| baseline levels of acetamide in milk before treatment is initiated (ppm) | 0.34 ± 0.08 | 0.34 ± 0.08 | 0.25 ± 0.05 | 0.25 ± 0.05 |
| average level of acetamide in milk (ppm) | 6.37 ± 2.41 | 9.59 ± 5.09 | 4.11 ± 1.48 | 5.74 ± 2.92 |
| median level of acetamide in milk (ppm) | 6.19 | 8.40 | 4.33 | 6.14 |
| acetamide trend (ppm/day) | 0.081 | −0.054 | −0.104 | −0.116 |
| p-value for acetamide trend | 0.245 | <0.001 | 0.002 | <0.001 |
| milk yield (kg/day) | 6.11 ± 1.68 | 6.71 ± 1.79 | 7.76 ± 2.43 | 9.93 ± 2.42 |
| AFEX feed intake (kg/day) | 2.91 ± 0.58 | 6.27 ± 1.36 | 3.22 ± 0.61 | 5.87 ± 1.30 |
| acetamide in milk as a percentage of acetamide intake (%) | 0.27 ± 0.13% | 0.21% ± 0.14% | 0.20% ± 0.12% | 0.19% ± 0.13% |

“Amount of acetamide present in milk in experimental animals prior to introduction of AFEX pellets. Average concentration of acetamide in milk for 3 (cattle) or 4 (buffalo) weeks on AFEX diet following the 14 days of initial transition to the AFEX diet. Linear trend in the change of acetamide over time during the 3 or 4 weeks after the initial transition to the AFEX diet. Acetamide present in the milk as a fraction of the amount present in the AFEX pellets in the feed.

Table 4. Comparison of Acetamide Concentration Immediately after Reducing AFEX Pellet Intake and after Eliminating AFEX Pellet Intake

| 2 days after 50% reduction of pellets in diet | 7 days after removal of pellets from the diet |
|---------------------------------------------|---------------------------------------------|
| conc (ppm) | % reduction | conc (ppm) | % reduction | % of baseline |
| Trial 1 low | 1.41 ± 0.60 | 78.5% | 0.34 ± 0.12 | 94.7% | 98.0% |
| Trial 1 high | 1.34 ± 0.46 | 82.0% | 0.32 ± 0.10 | 95.7% | 95.8% |
| Trial 2 low | 1.23 ± 0.23 | 65.6% | 0.15 ± 0.03 | 95.7% | 58.2% |
| Trial 2 high | 1.83 ± 0.95 | 64.1% | 0.12 ± 0.02 | 97.1% | 51.9% |

“Comparison of acetamide concentration to the average concentration in the milk of animals over the last 2 weeks of treatment. Comparison of acetamide concentration to the average concentration in the milk of animals prior to introduction of AFEX pellets. All values listed are listed as mean ± standard deviation.

(represented by the dashed lines). All five animals that were fed at 30% inclusion on day 8 showed a transient peak of acetamide concentration in milk. In contrast, only one out of six animals on the 3% transition diet and two out of six animals on the 10% transition diet produced an acetamide concentration peak once AFEX inclusion was increased to 30% of the diet. During the transition week, animals on the 3% diet tended to have lower acetamide levels in the milk (P < 0.08) than those in the 10% diet.

While further testing is necessary with different ruminant species and breeds, these findings are indicative that a feeding regimen can be developed to mitigate any peaks in milk acetamide levels when AFEX pellets are first incorporated in the animals’ diet.

Concentration of Acetamide in Milk after Adapting to AFEX Diets. In the 3–4 weeks following the initial transition to AFEX diets, the milk from lactating buffalo and cattle in Trials 1 and 2 contained a significantly (p < 0.01) higher concentration of acetamide than during the period before introducing AFEX to the diet, as summarized in Table 3. At high (50%) incorporation of AFEX pellets, acetamide concentrations in cattle milk samples averaged 5.7 ppm and buffalo milk samples averaged 9.6 ppm, which are higher than the 0.2–0.4 ppm measured in milk samples when on the control diet. The control diets did not contain detectable acetamide, suggesting that the acetamide measured in the milk during this time may be an endogenously produced metabolite. Median milk values are also shown in the table, as these values are used by the Joint FAO/WHO Expert Committee on Food Additives to determine the estimated daily intake in setting safety standards.22

A significant difference (p = 0.01) was observed between the average milk acetamide levels of Murrah buffaloes fed the low and high AFEX treatments (6.19 versus 8.40 ppm) for 3 weeks following initial transition. However, the milk acetamide levels in cattle fed the low AFEX and high AFEX diets in the 2 weeks following initial transition were not significantly different. When feed intake and milk production are both taken into consideration, the fraction of acetamide present in milk as a function of that ingested in the feed is largely similar across all four treatments as shown in Table 3. Even with the higher concentration of acetamide observed in the milk relative to the baseline, the acetamide excreted in milk represents only ~0.2% of the total acetamide ingested by the animals. While no other excretions (breath, urine, or fecal matter) were tested for acetamide, the hypothesis is that the vast majority of the remaining 99.8% acetamide is metabolized within the animal, likely by rumen microorganisms.28

There is a slight but statistically significant (p < 0.01) trend of milk acetamide concentrations decreasing over the 3 week time period following transition to AFEX diets for cattle (Trial 1). There is also a trend for decreasing acetamide (p = 0.08) for buffalo (Trial 2). The continued decrease in milk acetamide suggests that the rumen may continue to adapt to more completely metabolize acetamide while animals are on an AFEX diet, beyond the initial adaptation that occurs when AFEX pellets are first introduced into the animal diet. Longer feeding trials are needed to confirm this continuous adaptation as well as to determine if a steady-state acetamide concentration in milk for both cattle and buffalo exists and what that concentration might be.

Decrease in Milk Acetamide Concentration When AFEX Pellets Are Withdrawn from the Diet. At the conclusion of Trials 1 and 2, AFEX pellets were reduced in the feed by 50% for 2 days before being eliminated from the diet completely. Acetamide concentration in milk was measured on
the second day of the 50% reduction as well as 1 week after the complete removal of AFEX pellets. These concentrations were compared to the average concentration of acetamide in milk for each animal over the last 2 weeks of the treatment period as well as the initial baseline, as shown in Table 4.

Milk acetamide concentrations dropped rapidly with a decrease in AFEX pellets in the diet. The acetamide concentration decreased by 65% in the case of the cattle and 80% in the case of the buffalo within 2 days of reducing AFEX pellets in the feed by 50%. A significant difference in the extent of reduction during this 2 day period was observed between the buffalo and the cattle ($p < 0.01$) but not between the low and the high AFEX treatments for either species ($p = 0.99$). The acetamide concentration returned to a value at or below the baseline within 1 week after the removal of AFEX pellets from the diet with a significant difference ($p < 0.01$) between the cattle and the buffalo.

These observations are consistent with the findings of Putcha et al.,$^{29}$ who found that acetamide was excreted in the urine with a half-life of approximately 16 h in rats. Adam et al. also dosed sheep with labeled acetamide directly in the rumen and noted that peak urine accumulation of 15N occurred after only 8 h.$^{30}$ Furthermore, the acetamide appeared to be virtually eliminated from the rumen within 15 h in that study, which is also consistent with our findings of rapid reduction of acetamide from milk following removal from the diet. Likewise, labeled carbon from acetamide fed to sheep declined in the rumen with a half-life of 2.5 h.$^{31}$ Additional studies will be necessary to further delineate the metabolism and excretion of acetamide in large ruminant animals.

**Presence of Acetamide in Beef.** The concentrations of acetamide in the meat of Trials 4 and 5 are shown in Figure 3.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Amount of acetamide (in parts per billion) found in meat of cattle fed an AFEX-rich diet or control diet for 160 days (Trial 4) or calves fed an AFEX-rich or control diet for 50 days (Trial 5). Error bars represent the standard deviation of 12 (Trial 4) or 9 (Trial 5) samples. Acetamide concentration in meat purchased from the market is also shown as reference; error bars represent the standard deviation of 44 samples. Red diamond represents the median for each treatment.

In Trial 4, 12 steers were fed AFEX pellets at 30% of their total diet for 160 days. Animals were slaughtered the morning after the last day of consuming pellets, and the meat was tested for acetamide. The control consists of 12 animals fed no AFEX pellets. In Trial 5, 8 male calves were fed AFEX pellets at 50% of the diet for 50 days before immediate slaughter. No significant difference was observed in the acetamide concentration between the control and the treatment samples in either of the two trials. The steer values were in a range similar to marketed beef samples, while the calf acetamide was approximately twice as high as that observed in the steer study. It is unclear why the calf values were higher than in steers, but it is unlikely to be due to AFEX pellets as the control values are also higher.

Only a terminal data point was obtained from the two beef cattle studies. The most likely explanation for the absence of increased acetamide in beef is that rumen adaptation continued for a prolonged time period and by 50–160 days acetamide levels in tissues had decreased to that in controls. It is also possible that the brief overnight starvation contributed to some of the decline in acetamide levels in muscle tissues or that acetamide is preferentially stored within the animal in locations other than meat. Additional investigation is necessary to determine whether other ruminants (such as sheep and goats) will respond in the same manner as these beef cattle.

**Implications for Future Development.** While it has long been known that acetamide is a byproduct formed during AFEX treatment,$^{20}$ our recent findings indicate that, during pilot-scale AFEX production, the acetamide produced is not only fairly reproducible across multiple batches but also consistent across multiple types of crop residues. As AFEX technology is scaled up and adopted, acetamide will become a more commonly encountered feed contaminant in AFEX-treated cereal crop residues.

Both cattle and buffalo responded to the presence of acetamide in AFEX feeds by adapting their rumens. A transient spike in acetamide is highly likely to be measured when AFEX feed is rapidly introduced to animals, for example by a step change or even a linear daily increase over 1 week. Our findings suggest that an adaptation period at low AFEX inclusion (3% of the total diet) is sufficient to mitigate this transient spike in acetamide concentration. Further studies are needed to confirm this approach, understand the variance within animals and within species, and develop it into a protocol that can be practiced by farmers.

Feeding AFEX-treated crop residues to cattle resulted in increased acetamide concentrations in milk. In the 3 (cattle) and 4 (buffalo) weeks following the initial transition, concentrations of acetamide in milk were 16–23 times higher in cattle than before AFEX pellets were introduced to the diet and 19–28 times higher in buffalo. It is possible that acetamide levels in milk may decrease over time due to continued rumen adaptation, a phenomenon that is worthy of additional investigation. Acetamide must thus be considered a food contaminant as well as a feed contaminant.

While studies finding acetamide in human foods have periodically been published since the 1980s, it is only recently that acetamide has been recognized as a human food contaminant. Bercu et al. estimates that human exposure to acetamide in the United States is $\sim 1.5$ mg/day based on exposure from milk, meat, eggs, and coffee.$^{31}$ While beef from animals consuming AFEX pellets is not likely to change human exposure as shown in Figure 3, milk from animals consuming AFEX pellets could substantially increase this exposure. Currently, no regulatory agencies have provided acceptable daily intake guidelines for acetamide; the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifically declined to make such a ruling in 2005.$^{32}$ Research is currently underway to assess genotoxicity as well as to determine an acceptable daily intake level. Investigations and risk assessment are needed to determine whether acetamide at the levels
currently encountered in foods or likely to be encountered were AFEX technology to be adopted poses a substantial cancer risk to humans. Additional investigations are also needed to determine if our findings for both meat and dairy production are consistent across species, breeds, and different growth periods.

Availability of sufficient feed for ruminants, particularly in the developing world, will continue to be a prominent challenge as the size of the middle class increases. Chemical treatment of straw such as AFEX treatment has great potential to increase the availability of nutritious feeds while simultaneously reducing air pollution due to the alternative use of crop residues. Technologies such as AFEX can only be adopted if the human health risk of feed and food contaminants such as acetamide is found to be acceptably low by relevant regulatory agencies.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/ac5040300.

Raw data of acetamide concentration in milk for buffalo on the low AFEX diet during the 5 week feeding trial in Karnal, India; raw data of acetamide concentration in milk for buffalo on the high AFEX diet during the 5 week feeding trial in Karnal, India; raw data of acetamide concentration in milk for cattle on the low AFEX diet during the 4 week feeding trial in Karnal, India; raw data of acetamide concentration in milk for cattle on the high AFEX diet during the 4 week feeding trial in Karnal, India (PDF)

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Notes

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REFERENCES

(1) Bruinsma, J. World Agriculture: Towards 2015/2030: an FAO Perspective; FAO/Earthscan: London, 2003.
(2) Food and Agricultural Organization of the United Nations. The State of Food and Agriculture: Livestock in the Balance; FAO: Rome, 2009.
(3) Lohan, S. K.; Jat, H. S.; Yadav, A. K.; Sidhu, H. S.; Jat, M. L.; Choudhary, M.; Peter, J. K.; Sharma, P. C. Burning issues of paddy residue management in north-west states of India. Renewable Sustainable Energy Rev. 2018, 81, 693–706.
(4) Van Soest, P. J. Rice straw, the role of silica and treatments to improve quality. Anim. Feed Sci. Technol. 2006, 130, 137–171.
(5) Campbell, T. J.; Teymouri, F.; Bals, B.; Glassbrook, J.; Nielson, C. D.; Videto, J. A packed bed ammonia fiber expansion reactor system for pretreatment of agricultural residues at regional depots. Biofuels 2013, 4, 23–34.
(6) Bals, B.; Murmen, H.; Allen, M.; Dale, B. Ammonia fiber expansion (AFEX) treatment of eleven different forages: Improvements to fiber digestibility in vitro. Anim. Feed Sci. Technol. 2010, 155, 147–155.
(7) Griffith, C. L.; Ribeiro, G. O.; Oba, M.; McAllister, T. A.; Beauchemin, K. A. Fermentation of Ammonia Fiber Expansion treated and untreated barley straw in a rumen simulation technique using rumen inoculum from cattle with slow versus fast rate of fiber disappearance. Front. Microbiol. 2016, 7, 1839.
(8) Blummel, M.; Teymouri, F.; Moore, J.; Nielson, C.; Videto, J.; Kodukula, P.; Potthu, S.; Devulapalli, R.; Varjiakshapanicker, P. Ammonia Fiber Expansion (AFEX) as spin off technology from 2nd generation biofuel for upgrading cereal straws and stovers for livestock feed. Anim. Feed Sci. Technol. 2018, 236, 178–186.
(9) Mor, P.; Bals, B.; Tyagi, A. K.; Teymouri, F.; Tyagi, N.; Kumar, S.; Bringi, V.; VandeHaar, M. Effect of ammonia fiber expansion on the available energy content of wheat straw fed to lactating cattle and buffalo in India. J. Dairy Sci. 2018, 101, 7990–8003.
(10) Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide/IARC Working Group on the Evaluation of Carcinogenic Risks to Humans; IARC: Lyon, France, 1999; pp 1211–1221.
(11) Belasco, L. J. New nitrogen feed compounds for ruminants—a laboratory evaluation. J. Anim. Sci. 1954, 13, 601–610.
(12) Mahenthiralingam, E.; Draper, P.; Davis, E. O.; Colston, M. J. Cloning and sequencing of the gene which encodes the highly inducible acetamidase of Mycobacterium smegmatis. J. Gen. Microbiol. 1993, 139, 575–583.
(13) Simon, O.; Munchmeyer, R.; Gorsch, R.; Bergner, H. Einfluß von Harnstoff, Azetamid und Azetylhaarnstoff auf die freien Aminosäuren des Blutplasmas und die Aminosaurezusammensetzung der Pansenproteine beim Schaf. Arch. Tierernaehr. 1973, 23, 555–566.
(14) Kijora, C.; Gorsch, R.; Muller, J.; Bergner, H. Untersuchungen zum Umsatz von 14C-15N-Azetamid bei Schafen. Arch. Tierernaehr. 1975, 25, 165–181.
(15) Vismeh, R.; Haddad, D.; Moore, J.; Nielson, C.; Bals, R. D.; Campbell, T.; Julian, A.; Teymouri, F.; Jones, A. D.; Bringi, V. Assessment of exposures to acetamide in milk, beef, and coffee using xanthoxyl derivatization and GC/MS. J. Agric. Food Chem. 2018, 66, 298–305.
(16) Wei, F.; Furihata, K.; Zhang, M.; Miyakawa, T.; Tanokura, M. Use of NMR-based metabolomics to chemically characterize roasting process of chicory root. J. Agric. Food Chem. 2016, 64, 6459–6465.
(17) Sarks, C.; Bals, B. D.; Wynn, J.; Teymouri, F.; Schwegmann, S.; Sanders, K.; Jin, M.; Balan, V.; Dale, B. E. Scaling up and benchmarking of ethanol production from pelletized pilot scale AFEX treated corn stover using Zymomonas mobilis 8h. Biofuels 2016, 7, 253–262.
(18) Hanchar, R. J.; Teymouri, F.; Nielson, C. H.; McCalla, D.; Stowers, M. D. Separation of glucose and pentose sugars by selective enzyme hydrolysis of AFEX-treated corn fiber. Appl. Biochem. Biotechnol. 2007, 136–140, 313–325.
(19) Blummel, M.; Steele, B.; Dale, B. E. Opportunities from second-generation biofuel technologies for upgrading lignocellulosic biomass for livestock feed. *CAB Rev.* 2014, 9 (041), 1–8.

(20) Chundawat, S. P.; Vismeh, R.; Sharma, L. N.; Humpula, J. F.; da Costa Sousa, L.; Chambliss, C. K.; Jones, A. D.; Balan, V.; Dale, B. E. Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and dilute acid pretreatments. *Bioresour. Technol.* 2010, 101, 8429–8438.

(21) Sluiter, J. B.; Ruiz, R. O.; Scarlata, C. J.; Sluiter, A. D.; Templeton, D. W. Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods. *J. Agric. Food Chem.* 2010, 58, 9043–9053.

(22) Idaho National Laboratory. *Biomass Feedstock Library: Analysis Summary*; 2019; https://bioenergylibrary.inl.gov/Research/AnalysisSummary.aspx (accessed June 10, 2019).

(23) Mokomele, T.; da Costa Sousa, L.; Bals, B.; Balan, V.; Goosen, N.; Dale, B. E.; Gorgens, J. F. Using steam explosion or AFEX to produce animal feeds and biofuel feedstocks in a biorefinery based on sugarcane residues. *Biofuels, Bioprod. Biorefin.* 2018, 12, 978–996.

(24) Latham, E. A.; Anderson, R. C.; Pinchak, W. E.; Nisbet, D. J. Insights on alterations to the rumen ecosystem by nitrate and nitrocompounds. *Front. Microbiol.* 2016, 7, 228.

(25) Parish, T.; Mahenthiralingam, E.; Draper, P.; Davis, E. O.; Colston, M. J. Regulation of the inducible acetamidase gene of *Mycobacterium smegmatis*. *Microbiology* 1997, 143, 2267–76.

(26) Bergner, H. Metabolism of 14C- and 15N- labelled acetamide and acetylurea as NPN sources. *Can. J. Anim. Sci.* 1984, 64 (5), 37–38.

(27) Mor, P.; Bals, B.; Kumar, S.; Tyagi, N.; Reen, J. K.; Tyagi, B.; Choudhury, P. K.; Tyagi, A. K. Influence of replacing concentrate mixture with AFEX pellets on rumen fermentation, blood profile and acetamide content in the rumen of crossbred (Alpine × Beetle) female goats. *Small Ruminant Res.* 2019, 170, 109–115.

(28) Joint FAO/WHO Expert Meeting on Dietary Exposure Assessment Methodologies for Residues of Veterinary Drugs: Final Report; World Health Organization: Rome, Italy, 2012; p S.

(29) Putcha, L.; Griffith, D. P.; Feldman, S. Disposition of 14C-acetohydroxamic acid and 14C-acetamide in the rat. *Drug Metab. Dispos.* 1984, 12, 438–443.

(30) Adam, K.; Bergner, H.; Gorsch, R.; Kijora, C. Untersuchungen zum Umsatz von 14C-15N-Azetamid bei Schafen. *Arch. Tierernähr.* 1975, 25, 233–246.

(31) Bercu, J. P.; Galloway, S. M.; Parris, P.; Teasdale, A.; Masuda-Herrera, M.; Dobo, K.; Heard, P.; Kenyon, M.; Nicolette, J.; Vock, E.; Ku, W.; Harvey, J.; White, A.; Glowienke, S.; Martin, E. A.; Custer, L.; Jolly, R. A.; Thybaud, V. Potential impurities in drug substances: Compound-specific toxicology limits for 20 synthetic reagents and by-products, and a class-specific toxicology limit for alkyl bromides. *Regul. Toxicol. Pharmacol.* 2018, 94, 172–182.

(32) *Pesticide Residues in Food; 2004 Evaluations. Part 1–Residues*; World Health Organization, 2005.