



Waste to Wealth: Recycling Agricultural Wastes As Feed Additives

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An important goal of feed additives in animal nutrition is to improve feed efficiency with a concomitant reduction in the cost of production and invariably increase animal protein. Previously, the use of antibiotics at sub therapeutic concentrations in livestock production was essential to improve digestibility and efficiency of feed utilization, including animal health and performance. But current restrictions on the use of antibiotics as feed additives have strengthened research for alternatives, and natural feed additives are viewed as novel alternatives. Onion peel has been reported as a food waste but with its high content of phenolic bioactive compounds, it could be a viable candidate as a natural feed additive. More scientific information and data are required to validate its potential as a feed additive in ruminant nutrition. The aim of the study was to screen naturally available agricultural waste as a feed additive to improve animal performance for increased productivity. Using the *in vitro* batch culture technique, we evaluated the effects of onion peel at 0, 2.5, 5, 7.5 and 10% inclusion levels on dry matter digestibility and estimated short chain volatile fatty acids (SCFA) of high concentrate and high forage diets at 6, 24 and 48 h. Compared with the control, onion peel at 2.5% inclusion level had a slight increase (2-6%) in dry matter digestibility at 6 and 24 h, with higher values in the high forage diet. Consequently, numerical differences in the estimated SCFA values (0.26-3.36 mmol) and (0.29-3.85 mmol) for 0 and 2.5% inclusion levels respectively, indicate that more energy will be provided and available for improved animal performance with onion peel inclusion as a feed additive. Although the various onion peel inclusion levels impacted dry matter digestibility at different time periods, 2.5% inclusion level had the highest dry matter digestibility. Further studies are recommended including combining onion peel with other plant nutraceuticals for potential synergistic effects.

Keywords: Feed Additives, Feed Efficiency, Antibiotics, Bioactive Compounds, Agricultural Waste, Plant Nutraceuticals

Introduction

By-products are generated in billions of metric tons from agriculture and food processing industries worldwide. These by-products are abundantly available and considered as the cheapest sources of biomass, which can be broadly divided into animal-derived and plant-derived by-products according to Bandara & Chalamaiiah (2019). However, these by-products need to be utilized for useful purposes to avoid pollution through waste. Therefore, it is crucial to explore and improve methods that can enhance utilization of agricultural by-products as value added products. Onion (*Allium cepa*) was reported as the second most important horticultural crop worldwide and commonly consumed globally with increased production in the past 20 years, consequently resulting in high waste accumulation from onion processing which could have detrimental effects on the environment by Chernukha et al. (2022) and Kumar et al. (2022). Thus, there is a need to develop sustainable agricultural processes that will encourage farmers to increase production through recycling of waste. Certain parts of onion waste were reported to be rich in flavonoids and

onion peel (OP) also known as onion skin is the richest according to Bedrníček et al. (2019). Hence, OP has been reported as a valuable source of phytochemicals, which are rich in bioactive phenolic compounds according to Albishi et al. (2013), Celano et al. (2021) and Kumar et al. (2022). Dried OP is a rich source of flavonoids and aglycone, along with anthocyanins which possess antioxidants depending on the variety. Flavonoids were reported as indispensable component in nutraceutical application with ability to improve gut health by Pei et al. (2020), and proposed as natural feed additives in ruminants according to Kumar et al. (2022), Olagaray & Bradford (2019), and Panche et al. (2016). Moreover, studies have shown flavonoids as an economic source of feed additives according to Albishi et al. (2013) and Chernukha et al. (2022). Albishi et al. (2013) reported six times more phenolic concentration in OP than their edible flesh counterparts and Kumar et al. (2022) confirmed OP as a more concentrated source of phytochemicals than the edible flesh. Additionally, Bayram (2019) and Kalantar (2018) reported flavonoids as polyphenolic compounds which have a similar pattern of action similar to monensin and other types of antibiotics. Hence, Kumar et al. (2022) reported OP as one of the important agricultural by-products rich in bioactive compounds that can be explored by researchers. However, Kalantar et al. (2018) recommended further studies to confirm the usefulness of flavonoids in animal diets, since antibiotic resistance has led to the search and exploration of natural alternative products that can be used in animal nutrition for efficient feed digestibility to improve performance according to Bayram (2019). Therefore, we hypothesized that OP inclusion at various levels in dairy diets could be a viable natural feed additive and the aim of the study was to explore naturally available agricultural waste as feed additive to improve animal productivity.

Materials And Methods

Animal Care and Feeding

All animal procedures and uses were approved by the North Carolina Agricultural and Technical State University Institutional Animal Care and Use Committee (IACUC). This study was conducted at North Carolina Agricultural and Technical State University Dairy Research and Training Facility (NCAT DRTF; Greensboro, NC). The cannulated cows were observed daily for health problems and treated according to routine management practices at the DRTF maintained under IACUC approved protocol 21-009.0.

Sample Preparation and Experimental Design

The OP was prepared from onion peels purchased from Boardman Foods, Inc., Boardman, OR. The yellow variety (*Allium cepa L.*) was used for this study. The OP was cleaned and air dried, then milled through a 2-mm sieve of Retsch miller (model SM 100; Retsch GmbH, Haan, Germany). Two dairy diets were collected from the NCAT dairy farm unit. The diets were high concentrate (HC) and high forage (HF). The diets were dried in a forced air oven (model Isotemp 180L; Thermo Fisher Scientific, Pittsburgh, PA) at 55°C and milled through a 2-mm sieve of Retsch miller (model SM 100; Retsch GmbH, Haan, Germany). About 100 g of HC and HF was measured for various OP inclusions using benchtop scale (model 403; Denver Instrument Co., Arvada, CO) and combined with the OP at various inclusion levels and thoroughly mixed with a waring blender mixer (model 51BL31; Waring Commercial Products, McCConnellsburg, PA). The OP inclusion levels were 0, 2.5, 5, 7.5 and 10% by weight in g. Approximately 0.5 g of the diets

were weighed with analytical scale (model VWR-224AC; VWR International, Radnor, PA) into Ankom bags (F57; Ankom Technology Corp, Macedon, NY) and sealed using an impulse sealer (model AIE 200; American International Electric, Inc., City of Industry, CA) with four replicates prepared for each treatment which were distributed into 100 mL bottles (Cat# 223747; Wheaton Science Products, Millville, NJ) according to treatment. The experimental design was a 2 x 3 x 5 factorial design with 4 replicates for each treatment.

In vitro Procedures

The effects of five OP inclusion levels were evaluated using the HC and HF diets. Dry matter digestibility and estimated short chain fatty acids (SCFA) were calculated at 6, 24 and 48 h in an *in vitro* batch culture. Rumen fluid (RF) was collected from 2 grazing lactating multiparous Holstein cows at mid lactation via the rumen cannula. The *in vitro* batch fermentation experiment was carried out with three replicates for each inclusion level at 6, 24, and 48 h for three consecutive days using the same 2 lactating multiparous Holstein cows. The *in vitro* procedure was based on the methods described by Anele et al. (2014) with some modifications. The RF from the cows were mixed and strained through 4 layers of cheesecloth. And the pH of the pooled rumen fluid (RF) sample was measured immediately using a benchtop pH meter (model B10P; VWR International, Radnor, PA). Aliquots of 15 mL of strained RF anaerobically flushed with CO₂ maintained at 39°C in a water bath (model WBE28; VWR International, Radnor, PA) were added to three 100 mL serum bottles (Wheaton Science Products, Millville, NJ) containing a previously CO₂ gassed 45 mL of McDougall's buffer (McDougall, 1948) and pre-warmed at 39°C in a water bath (model JAB18; Grant Instruments Ltd, Cambridge, UK). Bottles were capped with 20-mm rubber stoppers (Wheaton Science Products, Millville, NJ) and crimped with 20-mm aluminum caps (Wheaton Science Products, Millville, NJ). The *in vitro* batch fermentation was performed in a reach-in incubator (model 1915A; VWR International, Radnor, PA) at 39°C for 6, 24, and 48 h with an orbital shaker (model 3500; VWR International, Radnor, PA) at a speed of 125 rpm. In order to determine digestibility, sufficient dairy diets samples were ground with Retsch miller. Four Ankom bags (F57; Ankom Technology Corp, Macedon, NY) containing 0.5 g of the ground samples were weighed and sealed using a heat impulse sealer (Cat# MP-8 Intertek). Six blanks were included and four contained only rumen fluid. Buffer (without bags) was used to correct for background gas production and two contained 1 bag each (without sample) placed in the serum bottle for correction factor. Blanks were prepared for each time period according to experimental design.

Gas production was measured as pressure per square inch (PSI) in each bottle at each time period by inserting a BD 0.7 mm x 40 mm (Cat# 301000) hypodermic gauge needle attached to a manometer (model 33500-086; VWR International, Radnor, PA). The (Mauricio et al., 1999) equation: $y = B (1 - \exp - x [t - \text{lag}])$ was used to calculate volume estimated from the pressure values. In this equation 'y' represents the cumulative volume of gas produced at time 't' (h), 'B' represents the asymptotic gas volume, 'c' is the constant rate constant, and 'lag' serves as the time (h) between inoculation and commencement of GP. Gas produced per 100 mg of substrate by the equation $\text{ml per 100mg} = \text{ml gas}/(\text{mg of substrate}/100)$.

Once the 6, 24 and 48 h time periods were completed, the serum bottles were decapped using a decapping plier (Cat# C4020-101; Thermo Scientific, Rockwood, TN), and Ankom bags containing the dairy diets were washed with cold water and dried for 72 h at 55°C, after which dried bags were placed in a desiccator for 20 minutes and then weighed for dry matter digestibility

analysis. The short chain fatty acid (SCFA) was calculated using the equation $SCFA = 0.0239 \text{ GV (gas volume)} - 0.0601$ (Getachew et al., 2000).

Statistical Analysis

All data were analyzed using SAS version 9.4 SAS Institute, (2013).

Results And Discussion

Dry Matter Digestibility

The *in vitro* dry matter degradability (DMD) showed a wide range of digestibility in high concentrate and high forage diets evaluated across various inclusion levels at different time periods as presented in Table 1. The highest DMD value was observed at 2.5% inclusion level for 6 h time period, which ranged from 34.9% - 36.9% in HC and HF respectively, resulting in 2 - 6% increment in digestibility when compared with the control that ranged from 34.7% - 34.9% in HC and HF respectively. This is consistent with a previous study by Olagaray & Bradford (2019) that reported that quercetin aglycone was 90% degraded within 5 h of *in vitro* simulation study. The result was in accordance with scientific reports on flavonoids having potential to improve performance and production in ruminants by Kalantar (2018) and Olagaray & Bradford (2019). It was observed that digestibility in HF was better than HC at 6 h time period, which is expected to increase forage intake because a higher value of DMD is better. Our results are consistent with a previous report by Abu et al. (2018) who reported improved feed intake in ewes supplemented with 3% onion powder. However, a poor relationship between time and DMD was observed due to the highly variable digestibility of HC and HF at various OP inclusion levels. The study confirmed the statement by Kumar et al. (2022) that OP is one of the important agricultural by-products rich in bioactive compounds that can be explored to develop nutrient supplements.

Table 1. Effects of onion peel inclusion levels on dry matter digestibility of two dairy diets.

Variables	Inclusion Levels (%)					SEM	P-values
	Control	2.5	5.0	7.5	10.0		
6 h							

HC	34.70 ^{ij}	34.90 ⁱ	33.10 ^{ij}	31.80 ^j	27.20 ^k	1.255	<0.01
HF	34.90 ^{ij}	36.90 ⁱ	33.50 ^{ij}	33.70 ^{ij}	34.40 ^{ij}	1.255	<0.01
24 h							
HC	56.30 ^f	57.30 ^f	55.60 ^{fg}	55.50 ^{fg}	55.70 ^{fg}	1.255	<0.01
HF	49.70 ^h	50.80 ^h	52.00 ^{gh}	52.50 ^{gh}	50.30 ^h	1.255	<0.01
48 h							
HC	66.20 ^{abcd}	66.20 ^{abcd}	67.20 ^{abc}	69.70 ^a	68.30 ^{ab}	1.255	<0.01
HF	65.30 ^{bcd}	62.20 ^e	63.20 ^{cde}	64.20 ^{cde}	62.80 ^{de}	1.255	0.011

^{abcd} Values with unlike superscripts differ by $P < 0.05$ using Tukey's test

Short Chain Fatty Acid

Short chain fatty acids (SCFA) represent about 70 – 80% of energy absorbed by ruminants and both rates and proportions of individual SCFA affect energy supply. The highest estimated total SCFA over the 24 h was observed in 2.5% inclusion level with a range of 0.466 - 3.85 mmol and 0.288 - 2.85 mmol in HC and HF, respectively as shown in Table 2, whilst the control ranged from 0.477 - 3.36 mmol and 0.288 - 2.85 mmol in HC and HF, respectively. The result is similar to a previous study by Abu et al. (2018), who reported a significant increase in total volatile fatty acids (TVFA) in feed supplemented with onion for Ossimi ewes and concluded that onion as a feed additive can improve energy production and carbohydrate metabolism. In addition, Olagaray & Bradford (2019) reported that the degradation products of quercetin can be further metabolized to VFA. Therefore, the outcome of the study is in accordance with reports that suggested flavonoids to be used as feed additives to promote growth and development in ruminant animals instead of antibiotics Kalantar et al. (2018) and Olagaray & Bradford (2019). Additionally, Olagaray & Bradford (2019) reported that flavonoids can act as alternative carbon source for metabolism for rumen microbes, potentially resulting in greater TVFA which confirmed flavonoids as glucose derivatives of quercetin and kaempferol as reported by Albishi et al. (2013) and Oskoueian et al. (2013).

Table 2. Effects of onion peel inclusion levels on short chain fatty acids of two dairy diets.

Variables	Inclusion Levels (%)					SEM	P-values
	Control	2.5	5.0	7.5	10.0		
6h							

HC	0.47 ⁱ	0.46 ⁱ	0.46 ⁱ	0.49 ⁱ	0.46 ⁱ	0.250	<0.01
HF	0.26 ⁱ	0.28 ⁱ	0.29 ⁱ	0.24 ⁱ	0.24 ⁱ	0.250	<0.01
24h							
HC	3.35 ^f	3.84 ^e	3.29 ^{fg}	3.15 ^{fgh}	3.03 ^{gh}	0.250	<0.01
HF	2.68 ^h	2.85 ^{gh}	2.81 ^{gh}	2.85 ^{gh}	2.84 ^{gh}	0.250	<0.01
48h							
HC	6.98 ^{bc}	6.83 ^{bcd}	7.13 ^b	7.68 ^a	7.14 ^b	0.250	<0.01
HF	6.98 ^{bc}	6.93 ^{bcd}	6.25 ^{cd}	6.75 ^{bcd}	6.49 ^d	0.250	0.011

^{abcd} Values with unlike superscripts differ by $P < 0.05$ using Tukey's test

Conclusions

The rate and extent of digestibility including fermentation in the rumen are very important determinants for nutrients absorbed by ruminants, and the goal of a feeding program is to achieve an appropriate balance in available nutrients to meet the nutritional needs of animals. Therefore, in an effort to enhance productivity in ruminants since antibiotics cannot be used in animal production except for health issues, plant bioactives are being investigated as alternative feed additives and flavonoids which were reported to be present in onions has been recommended. The OP was confirmed to be rich in flavonoids, abundant in supply and can be easily accessible to farmers. The study showed the various onion peel inclusion levels impacted dry matter digestibility at different time periods, but 2.5% inclusion level exhibited better potential as an additive at a lower cost. Further studies are recommended including combining onion peel with other plant nutraceuticals for synergistic effects.

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