## ABSTRACT

LEATHERWOOD, WILLIAM LAWRENCE. Effect of Anthocyanins from Purple-fleshed Sweetpotatoes on *in vitro* Fermentation by Rumen Microbial Cultures. (Under the direction of Vivek Fellner).

Dairy cattle exposed to heat stress are prone to intracellular oxidative stress which can have negative effects on various aspects of their production. Anthocyanins are powerful antioxidants which have the potential to be used to help negate the deleterious effects of heat stress. Purple-fleshed sweetpotatoes (PFSP) contain high levels of anthocyanins and possess the possibility to be used as a feed source to help negate the effects of heat stress, provided they are not degraded by rumen fermentation and are absorbed by the body.

Three in vitro batch culture studies using rumen fluid from a non-lactating Holstein cow were developed to assess the effects that rumen fermentation has on the anthocyanin content of freeze dried PFSP powder and anthocyanin-rich PFSP extract. For each study, rumen fluid was collected, strained through double layered cheesecloth and mixed with artificial saliva buffer at a 2:1 ratio. Directly afterwards, 30ml of the rumen fluid-buffer mix were added to glass bottles containing the respective substrates, purged with $CO_2$ gas and capped with an airtight seal. Bottles were incubated in a water bath set at normal temperature of 37°C (NT) or high temperature of 41°C (HT) for predetermined time intervals. Upon removal from the water bath, bottles were placed on ice to cease fermentation. Anthocyanin, total phenolic, methane, volatile fatty acid, and pH measurements were taken for each bottle.

Three experiments were conducted. In Experiment 1, 1ml of anthocyanin extract and 1ml of 50% diluted extract were incubated with rumen fluid for 24hrs. The anthocyanin content of the extract was significantly reduced after the full incubation time for both levels.

In Experiment 2, three levels of freeze dried PFSP powder were incubated with rumen fluid: 1g, 5g, and 10g. The results revealed that incubation time had no effect on the anthocyanin content of the powder, regardless of amount. In Experiment 3, powder and extract treatments containing similar anthocyanin concentrations were incubated with rumen fluid. A control extract group incubated without rumen fluid was also added. The anthocyanin content was significantly reduced in the extract after 24hr in the bottles containing rumen fluid, but not in those without, indicating rumen fermentation was the cause of the reduction. There were no significant reductions in the anthocyanin content of the powder in Experiment 3.

These data imply that anthocyanins from PFSP extract are more susceptible to rumen fermentation than freeze dried powder. This is most likely due to the removal of starch and fiber from PFSP during the extraction process. In conclusion, it is possible for PFSP to be fed to dairy cattle during periods of heat stress, provided the anthocyanins would be absorbed by the body. Additional research is called upon to further investigate the behavior of anthocyanins and PFSP in ruminants.

# Effect of Anthocyanins from Purple-fleshed Sweetpotatoes on *in vitro* Fermentation by Rumen Microbial Cultures

by
William Lawrence Leatherwood

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Animal Science

Raleigh, North Carolina

2014

APPROVED BY:

| | |
|---|---|
| _____ | _____ |
| Vivek Fellner | Daniel H. Poole |
| Co-Chair | Co-Chair |

_____
Van Den Truong

## BIOGRAPHY

William was born on January 25, 1988 in Brevard, NC to Jim and Cindy Leatherwood. Here he was raised and graduated from Brevard High School in 2006. William attended NC State University and graduated in 2011 with B.S. degrees in Animal Science and Poultry Science. He began his Master's work in the fall of 2011 and is currently the assistant manager of the NC State University Dairy Farm.

## ACKNOWLEDGMENTS

I would like to thank Sarah Jo McLeod, Rong Reynolds, Vivek Fellner, Daniel Poole, and Den Truong for all of their help. I could not have done this without all of you.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# CHAPTER I

## Literature Review

### *Heat Stress in Dairy Cattle*

A common issue associated with the dairy cattle industry, especially in warm, temperate climates found in the southeastern United States, is heat stress and the negative effects that it can have on animal production. The upper ambient temperature limit for a Holstein cow to maintain a steady body temperature has been indicated as 25-26°C (~77-79°F) (Berman et al., 1985). It is not uncommon for temperatures in the southeast to remain above this limit for multiple days in a row, even at night. During these periods of elevated temperatures, dairy cattle are most susceptible to increases in their core body temperature, which can have debilitating effects on various aspects of their physiology and ultimately their production. These negative effects include, but are not limited to, reduced milk production, increased occurrence of clinical and subclinical mastitis, lower conception rates, higher rates of early embryonic death, and an increased delay in return to estrus (West, 2003). Many of these effects may be indirectly caused by reduced dry matter intake that accompanies hot weather, which causes a reduction in overall nutrient intake and absorption (Kadzere et al., 2002).

However, there are several direct physiological responses that arise as a result of heat stress. An increase in environmental temperatures causes an increase in body temperatures and a subsequent increase in the temperature of internal organs. This can lead to an increase in intracellular production of reactive oxygen species. These molecules are natural byproducts of the oxygen reduction process and are not harmful in low quantities. However,

if the level of reactive oxygen species becomes so high that natural reductants, or antioxidants, cannot balance them, there is increased stress at the cellular level, referred to as oxidative stress. The direct implications of oxidative stress include cell death, oxidative modification of macromolecules, and structural tissue damage (Lykkesfeldt & Svendsen, 2007).

*Use of Antioxidants in Heat Stress*

Several management techniques have been developed to help reduce the effects of heat stress in dairy cattle such as developing genetics to allow for more heat tolerance, changing the physical environment by adding shade, fans, sprinklers, etc., and improving nutrition (Beede & Collier, 1986). In terms of nutrition, the use of antioxidants has been proven as a successful technique. Various studies have presented evidence that in vitro blastocyst formation is enhanced by the addition of vitamin E, the most common lipid-soluble antioxidant in animal cells, and by other extracellular antioxidants such as anthocyanins when embryos are exposed to heat stress (Olson & Seidel, Jr, 2000; Sakatani, et al., 2007).

The effects of dietary antioxidant supplementation have also been studied in other species. Harsini et al. (2012) found that levels of superoxide dismutase, an enzyme that catalyzes the transformation of superoxide (a reactive oxygen species) into oxygen and hydrogen peroxide, were significantly higher in the skeletal muscle of broilers fed supplemental vitamin E and selenium under heat stress than the control group. Additional dietary vitamin E and selenium to these broilers under heat stress also reduced the

concentration of malondialdehyde in the skeletal muscle than the control group. Malondialdehyde is formed by the degradation of polyunsaturated lipids by reactive oxygen species and can cause toxic stress in cells.

The benefits exhibited by the use of antioxidants to treat oxidative stress are due to their ability to scavenge free radicals, reducing the total number of reactive oxygen species (Lykkesfeldt & Svendsen, 2007). Extracellular treatment with antioxidants can also aid in increasing intracellular antioxidants already present, such as glutathione, which further helps to protect cells from reactive oxygen species (Sakatani, et al., 2007).

*Description of Anthocyanins*

Anthocyanins are phenolic flavonoids present in a wide range of fruits and vegetables such as blueberries, grapes, and purple-fleshed sweetpotatoes (PFSP) as natural pigments. These compounds are water soluble, are absorbed easily into cells, are relatively resistant to changes in temperature and pH, and have been conveyed as having strong antioxidant activities (Harada et al., 2004). All of these characteristics make them notable candidates for use against oxidative stress.

While there are a large variety of anthocyanin structures, the core structure, called an anthocyanidin (illustrated below) is analogous to all varieties. The aromatic ring (A) is bonded to a heterocyclic ring (C) containing oxygen. This heterocyclic ring is connected by a carbon-carbon bond to another aromatic ring (B). The term anthocyanin refers to an anthocyanidin connected to sugars, or in its glycoside form (Castaneda-Ovando et al., 2009).

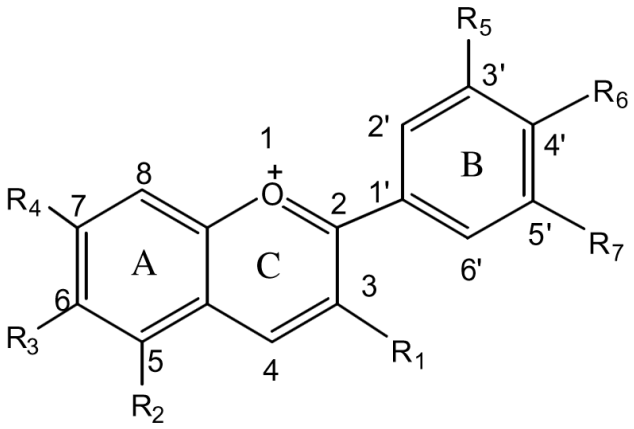



**Figure 1: Core Anthocyanin Structure (Anthocyanidin) (Castaneda-Ovando et al., 2009)**

The vast number of substituent compounds that may be bound to the core anthocyanidin give rise to a large variety of anthocyanins. The primary differences observed are the variety of of bonded sugars, the aromatic or aliphatic carboxylates that are bonded to the sugar(s), the position of these bonds, and the number of hydroxylated groups (Kong at al., 2003). As of 2006 there have been over 500 different anthocyanins and 23 anthocyanidins discovered (Andersen & Jordheim, 2006).

The stability of anthocyanins may become compromised when they are isolated and are thus extremely prone to degradation (Giusti & Wrolstad, 2001). Several factors contribute to the stability of anthocyanins including storage temperature, pH, concentration, light, chemical structure, oxygen, solvents, proteins, metallic ions, flavonoids, and presence of enzymes (Castaneda-Ovando et al., 2009).

The reducing nature of anthocyanins comes from their ability to donate phenolic hydrogen atoms from the anthocyanidin core in order to capture free radicals (Chen et al., 1996). Similar to the mechanism seen in catechols (Waterhouse, 2001), the anthocyanidin

can capture a free electron and stabilize itself through various resonance forms (Castaneda-Ovando et al., 2009). This mechanism of resonance and the molecular structure of the anthocyanidin allow it to remain very stable once it is oxidized. It can pass the free electron around without removing a hydrogen from another molecule (Figure 2).

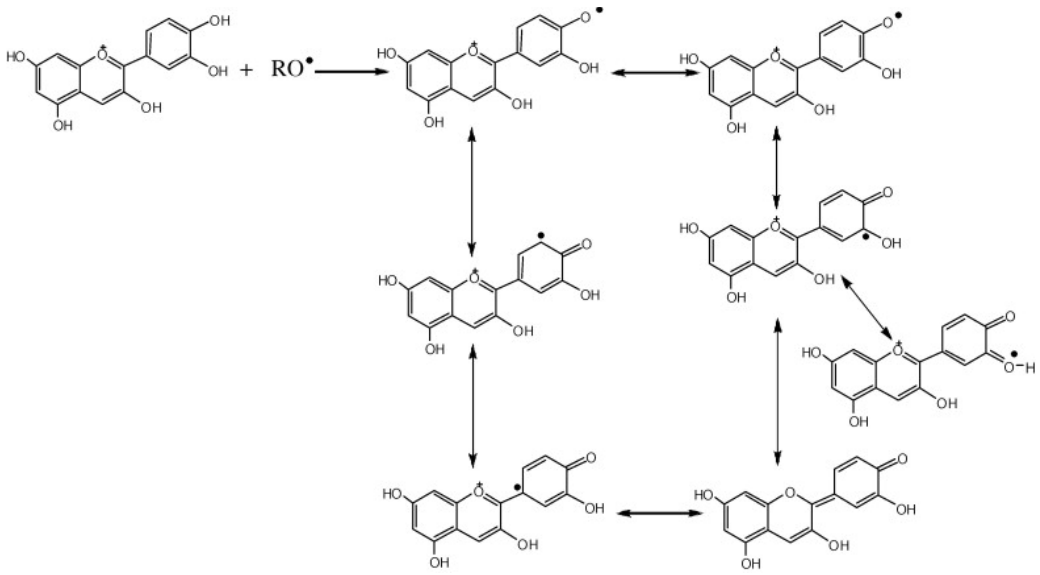


**Figure 2. Possible mechanism for anthocyanidin stabilization through resonance (Castaneda-Ovando et al., 2009)**

The antioxidant activity of anthocyanins from PFSP has been determined using several different methods and varies based on the part of the plant and the method of preparation. Using the DDPH assay, Steed and Truong (2008) determined the antioxidant activity of raw peels as 87.4 µmol TE/g fresh weight, which was almost twice as much as the puree (47.0 µmol TE/g fresh weight). The oxygen radical absorbance capacity (ORAC) values for the raw peels were 78.2 µmol TE/g fresh weight, compared to 26.4 µmol TE/g

fresh weight for the puree. These values are comparable to many other high-anthocyanin plants such as grapes, plums, and raspberries.

*Anthocyanins and Heat Stress*

Previous research indicates a high potential for the use of anthocyanins to treat heat stress in cattle. Sakatani et al. (2007) used anthocyanins from PFSP to help alleviate the negative effects that heat shock has on the intracellular redox status of bovine pre-implantation embryos. This in vitro study revealed that embryos treated with a small amount of anthocyanins (0.1μl/ml) had an increased blastocyst formation rate as well as decreased reactive oxygen species and increase glutathione levels. Higher anthocyanin treatment levels (1μg/ml and 10μg/ml) led to a decrease in reactive oxygen species and an increase in glutathione, but they had no effect on blastocyst formation.

Hosoda et al. (2012) demonstrated the benefits of feeding anthocyanin-rich corn to dairy cattle. Holstein cattle that were fed an anthocyanin-rich corn diet had significantly lower aspartate aminotransferase. This enzyme is a typical indicator of liver health- lower levels indicates a healthier liver. Cattle that received the anthocyanin-rich corn diet had also had significantly higher levels of this superoxide dismutase.

*Purple-fleshed Sweetpotatoes as Anthocyanin Source*

Anthocyanins are present in numerous fruits and vegetables. PFSP contain high levels of anthocyanins, which give them their distinct purple color, and represent a possible source of antioxidants to use in dairy cattle feed. In recent years, there has been an increased interest

in the potential marketing opportunities for PFSP in the United States (Truong, et al., 2010). This is primarily due to their health benefits and potential for food colorants. With the potential increase in PFSP production there is increased opportunity for the livestock industry to use refusals and byproducts of production or for growth strictly as a feedstuff source for animal consumption.

The level of anthocyanins present in the PFSP varies between varieties. The Stokes Purple-Fleshed Sweetpotato is one of the most common commercially grown varieties in the United States and can contain as much as 100mg anthocyanin per 100g fresh weight (Truong, et al., 2010). Methods of measuring anthocyanins and qualifying specific structures have been researched and developed extensively using HPLC and UV mass spectrometry (Giusti & Wrolstad, 2001).

Anthocyanins from PFSP have been reported to have stronger radical-scavenging activity than those present in red cabbage, purple corn, grape skin, or elderberry (Kano et al., 2005). PFSP anthocyanins are also more stable than those from some other sources due to acylation with hydroxylated aromatic organic acids (Bassa & Francis, 1987).

Extraction of anthocyanins from various fruits and vegetables has been studied extensively. The most common extraction procedures involve grinding and drying the raw products and soaking them in a polar solvent such as aqueous mixtures of methanol, ethanol, or acetone (Merken & Beecher, 2000; Kahkonen et al., 2001; Steed and Truong, 2008). Further purification practices may be necessary as other compounds such as organic acids and sugars may be co-extracted (Coutinho et al., 2004).

*Metabolism and Digestion of Anthocyanins*

There has been substantial research conducted assessing the behavior of anthocyanins and their biological antioxidant capacity, especially in monogastrics. Several studies have indicated that, when ingested orally, anthocyanins are absorbed by the stomach and gut of monogastrics and are eventually detected, structurally intact, in the blood (Passamonti et al., 2003; Miyazawa et al., 1999; Mazza et al., 2002).

Very little research can be found which directly utilizes anthocyanins in ruminant animals. One such study used anthocyanin-rich corn to evaluate the effects of rumen fermentation on anthocyanins. The results of this study indicate that anthocyanins in the corn remain very stable under rumen fermentation (Hosoda et al., 2009). This evidence, coupled with the fact that the abomasum and gut of ruminants have similar digestive and absorptive functions as the stomach and gut of monogastrics (Dijsktra at al., 2005), would lead to the assumption that, if anthocyanins are able to pass through the rumen of an animal without being degraded, they should be absorbed by the abomasum and gut. With this in mind, PFSP could be proper source of antioxidants that could be fed to cattle to help reduce the negative effects of heat stress.

## Literature Cited


Andersen, O., & Jordheim, M. (2006). The anthocyanins. In O. Andersen, & K. Markham, *Chemistry, biochemistry and applications* (pp. 452-471). Boca Raton, FL: CRC Press.

Bassa, I., & Francis, F. (1987). Stability of anthocyanins from sweet potatoes in a model beverage. A research note. *Journal of Food Science*, 1753-1754.

Beede, D., & Collier, R. (1986). Potential nutritional strategies for intensively managed cattle during thermal stress. *Journal of Animal Science*, 543-554.

Berman, A., Folman, M., Kaim, M., Mamen, M., Herz, Z., Wolfenson, D., . . . Graber, Y. (1985). Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical climate. *Journal of Dairy Science*, 1488-1495.

Castaneda-Ovando, A., Pacheco-Hernandez, M., Paez-Hernandez, M., Rodriguez, J., & Galan-Vidal, G. (2009). Chemical studies of anthocyanins: a review. *Food Chemistry*, 859-871.

Chen, Z., Chan, P., Ho, K., Fung, K., & Wang, J. (1996). Antioxidant activitiy of natural flavonoids is governed by number and location of their aromatic hydroxyl groups. *Chemistry and Physics of Lipids*, 157-163.

Coutinho, M., Quadri, M., Moreira, R., & Quadri, M. (n.d.). Partial purification of anthocyanins from Brassica oleracea (red cabbage). *Separation and Science Technology*.

Dijsktra, J., Forbes, J., & France, J. (2005). Introduction. In J. Dijsktra, J. Forbes, & J. France, *Quantitative aspects of ruminant digestion and metabolism* (pp. 1-10). Wallingford, UK: CABI Publishing.

Giusti, M., & Wrolstad, R. (2001). Characterization and measurement of anthocyanins by UV-Visible Spectroscopy. In *Current Protocols in Food Analytical Chemistry* (pp. F1.1.1-F.1.2.13). John Wiley & Sons, Inc.

Harada, K., Kano, M., Takayanagi, T., Yamakawa, O., & Ishikawa, F. (2004). Absorption of acylated anthocyanins in rats and humans after ingesting an extract of Ipomoea batatas Purple-Fleshed Sweetpotato tuber. *Bioscience, Biotechnology, and*

*Biochemistry*, 1500-1507.

Harsini, S., Habibiyan, M., Moeini, M., & Abdolmohammadi, A. (2012). Effects of dietary selenium, vitamin E, and their combination in growth, serum metabolites, and antioxidant defense system in skeletal muscle of broilers under heat stress. *Biological Trace Element Research*, 322-330.

Hosoda, K., Eruden, B., Matsuyama, H., & Shioya, S. (2009). Silage fermenative quality and characteristics of anthocyanin stability of anthocyanin-rich corn (Zea mays L.). *The Asian-Australasian Journal of Animal Science*, 528-533.

Hosoda, K., Eruden, B., Matsuyama, H., & Shioya, S. (2012). Effect of anthocyanin-rich corn silage on digestibility, milk production and plasma enzyme activities in lactating dairy cows. *Animal Science Journal*, 453-459.

Kadzere, C., Murphy, M., Silanikove, N., Maltz, & E. (2002). Heat stress in lactating dairy cows: a review. *Livestock Production Science*, 59-91.

Kahkonen, M., Hopia, A., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry*, 4076-4082.

Kano, M., Takayanagi, T., Harada, K., Makino, K., & Ishikawa, F. (2005). Antioxidative activity of anthocyanins from Purple-Fleshed Sweetpotato, Ipomoera batatas cultivar Ayamurasaki. *Bioscience, Biotechnology and Biochemistry*, 979-988.

Kong, J., Chia, L., Goh, N., Chia, T., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry*, 923-933.

Lykkesfeldt, J., & Svendsen, O. (2007). Oxidants and antioxidants in disease: oxidative stress in farm animals . *The Veterinary Journal*, 502-511.

Mazza, G., Kay, C., Cottrell, T., & Holub, B. (2002). Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *Journal of Agriculture and Food Chemistry*, 7731-7737.

Merken, H., & Beecher, G. (2000). Measurement of food flavonoids by high-performance liquid chromatography: A review. *Journal of Agricultural and Food Chemistry*, 577-599.

Miyazawa, T., Nakagawa, K., Kudo, M., Muraishi, K., & Someya, K. (1999). Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. *Journal of Agriculture and Food Chemistry*, 1083-1091.

Olson, S., & Seidel, Jr, G. (2000). Culture of in vitro-produced bovine embryos with vitamin E improves development development in vitro and after transfer to recipients. *Biology of Reproduction*, 248-252.

Passamonti, S., Vrhovsek, U., Vanzo, A., & Mattivi, F. (2003). The stomach as a site for anthocyanins absorption from food. *Federation of European Biochemical Societies Letters*, 210-213.

Sakatani, M., Suda, I., Oki, T., Kobayashi, S.-i., Kobayashi, S., & Takahashi, M. (2007). Effects of Purple-Fleshed Sweetpotato anthocyanins on development and intracellular redox status of bovine preimplantation embryos exposed to heat shock. *Journal of Reproduction and Development*, 605-614.

Steed, L., & Truong, V.-D. (2008). Anthocyanin content, antioxidant activity, and selected physical properties of flowable purple-fleshed sweetpotato purees. *Journal of Food Science*, 215-221.

Truong, V.-D., Deighton, N., Thompson, R., McFeeters, R., Dean, L., Pecota, K., & Yencho, C. (2010). Characterization of anthocyanins and anthocyanidins in purple-fleshed sweetpotatoes by HPLC-DAD/ESI-MS/MS. *Journal of Agricultural and Food Chemistry*, 404-410.

Waterhouse, A. (2001). The phenolic wine antioxidants. In E. Cadenas, & L. Parcker, *Handbook of Antioxidants* (pp. 401-406). New York: Marcel Dekker.

West, J. (2003). Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science*, 2131-2144.

# Chapter II

## The Effect of Anthocyanins from Purple-fleshed Sweetpotatoes on *in vitro* Fermentation by Rumen Microbial Cultures

### Introduction

Heat stress remains a common problem for dairy producers around the world, especially in warm, temperate climates during the summer months. When a cow is exposed to environmental temperatures above 25-26°C, it is unable to maintain a steady body temperature (Berman, et al., 1985). With increased body temperatures comes the potential for increased oxidative stress brought on by the increased production of reactive oxygen species. These free radicals give rise to an intracellular imbalance which can cause decreased milk production, negative effects on reproduction, and increased disease such as mastitis (Lykkesfeldt & Svendsen, 2007). Various methods of coping with increased ambient temperatures have been developed in order to help alleviate the negative effects of heat stress (Beede & Collier, 1986).

Some research shows that a possible aid in combating oxidative stress is the addition of antioxidants to the diet (Olson & Seidel, Jr, 2000; Sakatani et al., 2007). Antioxidants scavenge reactive oxygen species and help restore the intracellular balance between oxidants and reductants. Anthocyanins have long been known to have powerful antioxidant characteristics. One source of anthocyanins is the purple-fleshed sweetpotato (PFSP). Recent interest in developing new marketing opportunities for these plants gives rise to the possibility of using them as feed for livestock in order to help alleviate the negative effects of

heat stress (Truong, et al., 2010). Anthocyanins from PFSP, and other sources, have been shown to be absorbed by the stomach and intestines of monogastrics (Passamonti et al., 2003; Miyazawa et al., 1999; Mazza et al., 2002). Since the functions of digestion and absorption in the abomasum and gut of ruminants are similar to those of the stomach and gut of monogastrics (Dijkstra et al., 2005), it would imply that, if anthocyanins can pass through the rumen without being affected by the fermentation that occurs, they could pass through to the rest of the digestive system and be absorbed by the animal. The objective of this study was to assess the effects that rumen fermentation has on the anthocyanin content of PFSP.

## Materials and Methods

*Batch Culture Procedure*

For each experiment, approximately two liters of rumen fluid were collected from a non-lactating, fistulated Holstein cow located at the North Carolina State University Dairy Farm. To increase microbial biodiversity, the cow was fed a predominately high quality forage diet for 10 days prior to collection. Rumen contents were placed in a pre-heated thermos and transported to the lab where they were strained into an Erlenmeyer flask through double layered cheesecloth. The strained rumen fluid was mixed with artificial saliva buffer in a 2:1 ratio of buffer: rumen fluid. Carbon dioxide gas was purged through rumen fluid at all times to maintain anaerobic conditions. All bottles were also purged with carbon dioxide gas to displace oxygen and 30mL of rumen/saliva inoculant was added to each bottle. The bottles were immediately sealed with caps containing a rubber septum and crimped. The 0hr bottles were immediately placed on ice to cease fermentation, covered to prevent light

exposure and refrigerated at 4°C until methane readings were taken. All other bottles were placed into one of two water baths set at normal temperature of 37°C (NT) or high temperature of 41°C (HT). After the respective incubation times these bottles were placed on ice, covered, and refrigerated at 4°C until methane readings were taken. Following methane analysis, the bottles were uncapped and pH readings were taken. The bottles were then stirred thoroughly and a 14mL representative sample was pipetted from each bottle and placed in 15mL plastic test tubes. The tubes were wrapped in aluminum foil and placed in a -80°C freezer until further analysis for anthocyanin and phenolic concentrations. An additional sample of 1mL was taken for VFA analysis.

*Substrate Preparation and Handling*

Purple-fleshed sweetpotatoes (cv. Stokes Purple) were purchased from Jones Farm (Bailey, NC). The harvested roots were cured at 30 °C, 85-90% relative humidity for 7 days, and stored at 13-16 °C, 80-90% relative humidity. The roots were hand washed and cut into 0.50 cm thick slices, quickly placed in a plastic container covered with cheese cloth, and immediately placed in the -80 °C freezer. The frozen samples were freeze-dried using a VirTis Genesis 25XL freeze-dryer (Gardiner, NY) operated at -35 °C to -40 °C. The freeze-dried samples were weighed and ground into powders using a Mr. Coffee precision coffee grinder (Sunbeam, Boca Raton, FL), placed in sample bottles, and kept in -80 °C storage.

For anthocyanin extracts, 30 grams of freeze-dried powder was mixed with 500 ml of 0.1% trifluoro acetic acid (TFA) in methanol for 10 min. The slurry was settled for 1hr and the supernatant was decanted. The extraction was repeated for 3 more times and the collected

supernatants were combined. A TurbovapII evaporator (Zymark Corp., Hopkinton, MA) was used to evaporate the solvent under nitrogen at 4˚C and to concentrate the extract until a thick syrupy substance occurred. The concentrated anthocyanin extract was mixed with water to a final volume of 50ml and stored in -80˚C freezer until use.

The day before each experiment, the appropriate substrates were removed from the freezer, wrapped in aluminum foil and placed in a plastic cooler when transported to the Animal Science Lab. Substrates were placed in a -20°C freezer to reduce exposure to light and heat.

For experiments where the extract was used, the extract was placed in warm water to thaw the day of the experiment and added to the bottles while the rumen fluid and buffer were mixing. For experiments where the powder was used, the powder was weighed into the perspective bottles the day before the experiment. The bottles were placed in a cooler and stored overnight in a 4°C refrigerator. The day of the experiment, the bottles were placed in the lab at room temperature approximately 2 hours before inoculation with rumen fluid/buffer mix.

*Experiment 1*

Experiment one was comprised of two treatments at two incubation temperatures and two incubation times. The two treatments were 1mL of anthocyanin-rich liquid extract (PE) and 1mL of a 50% dilution of the extract (DE) from PFSP and the incubation times were 0 and 24 hours. The 24hr treatments were incubated at either NT or HT. All 0hr bottles were placed on ice immediately following inoculation with rumen fluid. All treatments were run in

duplicate. Blanks containing only rumen fluid and buffer were included at each time period and temperature. A total of 15 bottles (including blanks) were used in this experiment.

*Experiment 2*

A total of 55 bottles (including blanks) were used for experiment 2. There were 3 treatments with 3 incubation times and two temperature levels. The three treatments were 1g, 5g, and 10g, of freeze-dried PFSP powder (1GP, 5GP, 10GP respectively); the three time intervals were 0hr, 12hr, and 24hr; the 12 and 24hr bottles were incubated at either NT or NT. All treatments were run in triplicate. Blank bottles, or those containing only rumen fluid/saliva buffer, were added at each time and temperature level.

It is important to note that the 10GP bottles were nearly completely solid after incubation. The ratio of substrate:rumen media was likely too high and fermentation of the PFSP powder would not have been complete. Therefore, it was decided that this treatment be removed from analysis since any readings would be inaccurate.

*Experiment 3*

A total of 33 bottles were used for experiment three. There were three treatments with two incubation times and 2 temperature levels. In order to compare PFSP powder and extract, a similar anthocyanin concentration was needed. The anthocyanin concentration of the 0hr 1GP treatment was 48.7µg/ml. By multiplying this value by the total volume of the bottle (30ml) and dividing by the known concentration of the pure extract (890µg/ml) a level of 1.64mL extract was determined to be comparable to 1g of powder. Therefore, the three

treatments were 1g of freeze-dried PFSP (1GP), 1.64mL of PFSP liquid extract (E), and a control treatment with 1.64mL of the extract mixed with only the buffer solution (CE); the two incubation times were 0hr and 24hr; and the temperature levels were NT and NT. Only the 24hr treatments were divided into the two temperatures as the 0hr bottles were immediately placed on ice after inoculation with rumen fluid/saliva buffer. All treatments were run in triplicate. Blank treatments, or those containing only rumen fluid/saliva buffer were added at each time and temperature level.

*Methane and pH Analysis*

Using a 100μL Hamilton gas tight syringe, 10μL of gas was removed from each bottle. Gas samples were then immediately analyzed for methane using gas-liquid chromatography. Concentrations were reported based on the methane standard line. Once methane concentrations were determined the bottles were uncapped and pH was assessed using a pH meter.

*Volatile Fatty Acid Analysis*

A 2 mL aliquot of rumen culture was removed from each bottle and centrifuged at 15,000 rpm for 15 minutes. A 1 mL sample of the supernatant was pipetted out and placed in a microfuge tube with 200μL of meta-phosphoric acid internal standard (MIS) and centrifuged again at 15,000 rpm for 15 minutes. The supernatant was placed in a glass gas chromatography vial for subsequent analysis. Samples were analyzed for volatile fatty acids (VFA) by GLC (Model CP-3380; Varian, Walnut Creek, CA) with a NUKOL Fused Silica

Capillary Column of 30m x 0.25mm x 0.25*u*m film thickness (Supelco Bellefonte, PA). Peaks were identified based on retention times of standard VFA.

*Anthocyanin and Total Phenolic Analysis*

On the day of the anthocyanin and phenolic analysis, PFSP samples were removed from the -80°C freezer and taken to the Truong Lab in the Food, Nutrition and Bioprocessing Sciences department. Samples were allowed to thaw in warm water and mixed thoroughly before assays were performed.

The pH-differential method of measuring total monomeric anthocyanins as described by Giusti and Wrolstad (2001) was used to assess the anthocyanin levels in each bottle. First, the 14mL samples from each bottle were centrifuged for 30 minutes. Liquid was decanted and two dilutions were made, one with 0.025M potassium chloride at pH 1.0 and another with 0.4M sodium acetate at pH 4.5. Samples from Experiments 1 and 3 had dilution factors of 5. In Experiment 2, the higher level of PFSP powder (5g) required a higher dilution factor of 15 in order to obtain accurate absorbance readings. The absorbance of each diluted sample was measured at 530nm and 700nm and the blank used was distilled water. Total absorbance was calculated by subtracting the difference in the two readings at pH 4.5 from the difference in the two readings at pH 1.0:

$$Absorbance, A = (A_{530} - A_{700})_{pH1.0} - (A_{530} - A_{700})_{pH4.5}$$

After calculating the total absorbance, A, the following equation was used to determine the concentration of anthocyanins (mg/liter):

$$= (A \times MW \times DF \times 1000)/(\varepsilon \times l)$$

where MW is the molecular weight of anthocyanins (449.2 g/mol), DF is the dilution factor (5 or 15), ε is the molar absorptivity (26,900), and l is the path length (1 cm).

The procedure to determine the total phenolic concentration of each sample was derived from Singleton et al. (1999). The first step was to make a set of 1 mL standards to use in the final determination of the total phenolic concentration. These standards were made by combining 1 mM chlorogenic acid with distilled water to make chlorogenic acid concentrations between 0.1mM and 0.8mM. Then, 0.25 mL of all samples and standards were diluted with distilled water at a dilution factor of 20; 0.25 mL of 0.25N Folin and Ciocalteau's phenol reagent was added and allowed to react for 3 minutes; then 0.5 mL of 1N sodium carbonate solution was added and allowed to react for 1 hour. Absorbance levels were recorded for each sample at 725 nm with distilled water used as the blank (See table below for example).

**Table 1.** Example of plotting absorbance and concentration for total phenolic calculation

| **Standards** | |
|---|---|
| **Absorbance** | **Conc. (mM)** |
| 0.1047 | 0.1 |
| 0.1863 | 0.2 |
| 0.2862 | 0.3 |
| 0.364 | 0.4 |

The absorbance levels of the standards were plotted using Microsoft Excel with the standard concentration located on the x-axis and absorbance on the y-axis and a linear trend line was derived (y=mx+b) (See example below).

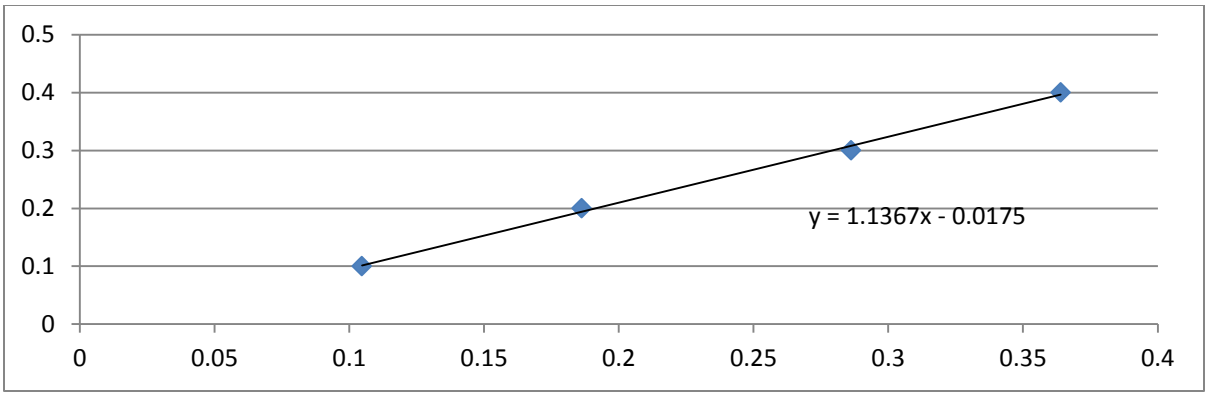



**Figure 3.** Example of graphing absorbance of standards for total phenolic calculations

The absorbance of each sample was plugged into the equation as the x value. The calculated y-value indicated the concentration of chlorogenic acid in mM. The chlorogenic acid concentration was converted to mg/ml by multiplying the concentration of chlorogenic acid by 0.3543mg/mol then multiplying by a dilution factor of 20. Concentration was then converted to µg/mol by multiplying by 1000.

*Statistical Analysis*

All blank values, except for pH, were subtracted from the measurements from the corresponding treatment data for respective times. This includes concentrations of methane, anthocyanins, phenolics, and all volatile fatty acids.

Statistical analyses were conducted using the PROC MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Data were analyzed as a repeated measures design. The model included pH, methane, anthocyanin, total phenolics, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and total VFA as the dependent variables and temperature, treatment and the interaction between temperature and treatment as the independent

variables. Least square means were calculated and significance was declared at $p<0.05$ with trends declared at $1.0>p>0.05$. The 0hr treatments were not included in these statistical analyses except for comparisons of anthocyanins and total phenolics.

Data on anthocyanin and total phenolic concentrations were analyzed separately using a repeated measures design. The model included anthocyanin and total phenolic concentrations as the dependent variables and treatment, time, and the interaction between treatment and time as the independent variables. The 0hr treatments were included in these analyses to illustrate the change in these variables over time. Another comparison was made with a repeated measures design with temperature, treatment, and temperature*treatment interaction as the independent variables, and anthocyanin and total phenolic concentrations as the dependent variables. The 0hr measurements were also included in these analyses.

## Results

*Experiment 1*

In an attempt to calculate a baseline treatment for which future experiments could be conducted, two levels of PFSP extract were chosen: 1mL of the extract (PE) and a 50% dilution of the same extract (DE). The anthocyanin and total phenolic concentrations for both treatments were significantly reduced after 24 hours of incubation.

Methane levels for both treatments at 0hr were between 15.37nmol/ml and 21.27nmol/ml (Table 2). The blank treatment at 0hr gave a methane reading of 19.19nmol/ml, indicating little to no fermentation, as expected (Table 2). At 24hr, methane levels increased to 712.55nmol/ml and 769.72nmol/ml for the NT and HT of PE respectively.

This is compared to the DE treatment levels of 428.31nmol/ml for NT and 678.49nmol/ml for HT (Table 5, Figure 4). Treatment effects on methane were not significant but were trending to be lower with the DE treatment. pH levels ranged between 6.1 and 6.4 for all treatments at 0hr and 5.85 and 6.05 for all treatments at 24hr (Table 5).

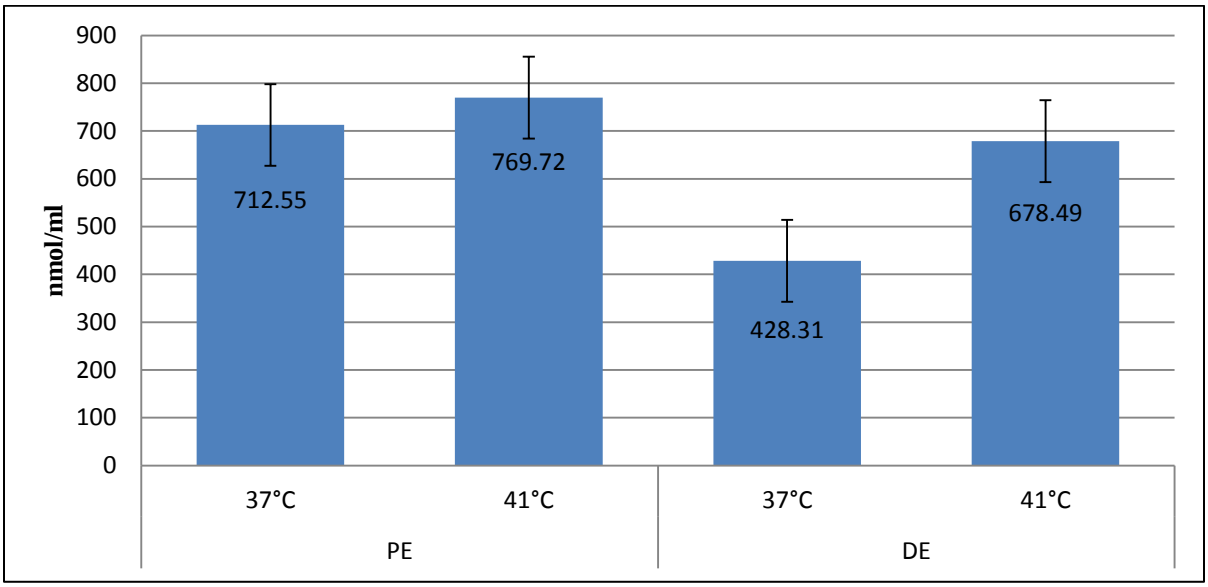



**Figure 4.** Methane concentrations after 24hr incubation in Experiment 1
PE=1ml PFSP Extract, DE=1ml 50% Dilution of PFSP Extract

Total VFA readings across substrate levels, indicated as mM, were between 20.78 and 24.41 at 0hr. At 24hr, the total VFA concentrations for NT and HT treatments of PE were 15.63 and 9.98mM, respectively (Table 5, Figure 5). The concentrations for DE were 7.85mM for NT and 5.05mM for HT (Table 5, Figure 5). All individual VFA trended to be higher in the PE verses the DE and trended to be higher in the NT bottles than the HT bottles. Treatment effects on propionate concentrations were the only significant effects of all VFAs.

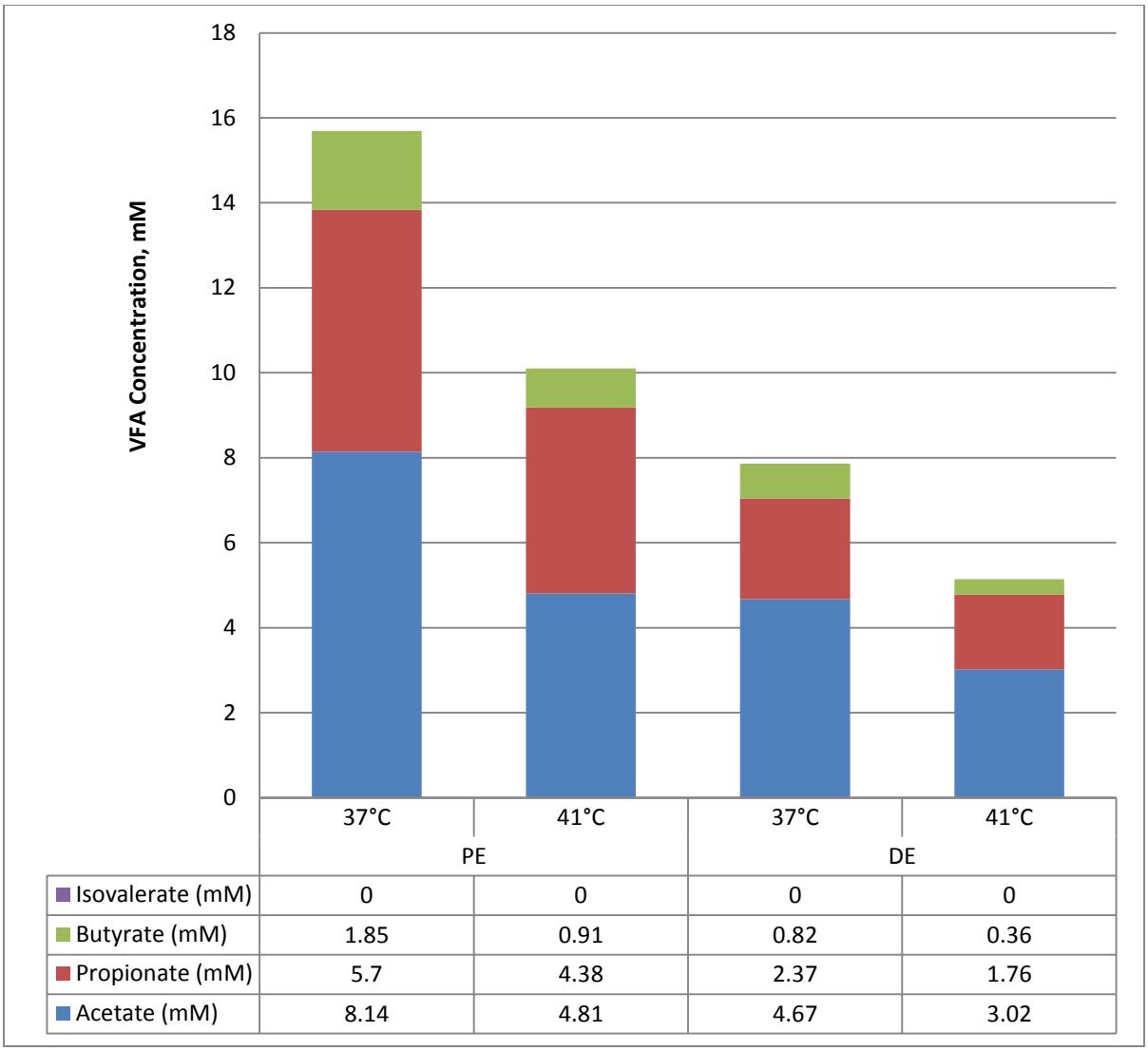



**Figure 5.** Volatile Fatty Acid concentrations after 24hr incubation in Experiment 1
PE=1ml PFSP Extract, DE=1ml 50% Dilution of PFSP Extract

Table 6 and Figures 6 and 7 show the comparison of anthocyanin and total phenolic concentrations between 0hr and 24hr. Anthocyanin concentration for PE decreased significantly from 4.59µg/ml to 0.229µg/ml over the 24hr period and from 2.12µg/ml to 0µg/ml for DE. Total phenolics for PE decreased significantly from 800µg/ml to 54.0µg/ml and decreased from 462µg/ml to 134µg/ml for DE.

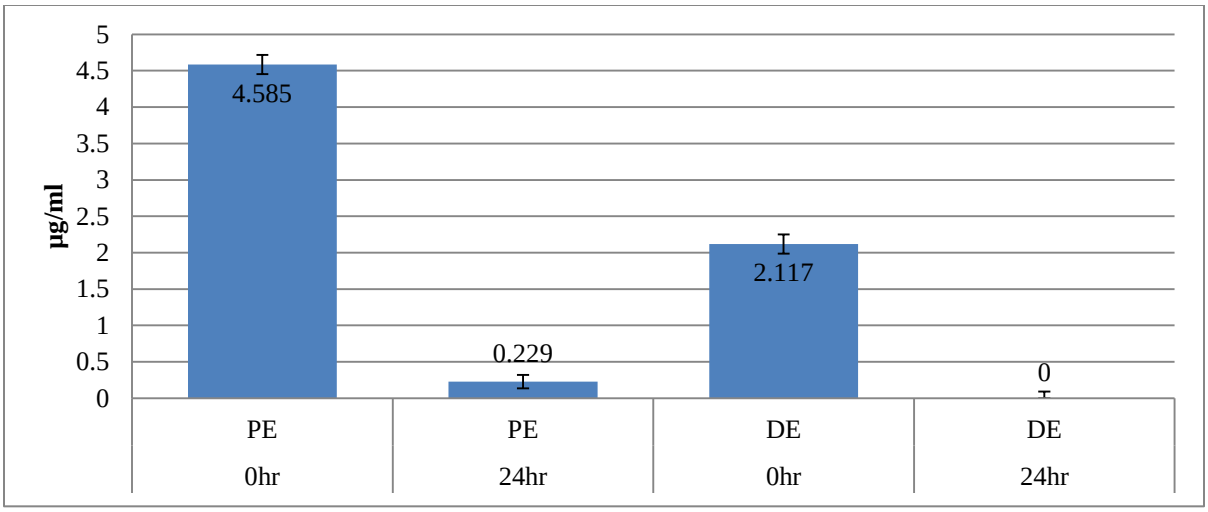



**Figure 6.** Change in anthocyanin concentration levels over time in Experiment 1
PE=1ml PFSP Extract, DE=1ml 50% Dilution of PFSP Extract

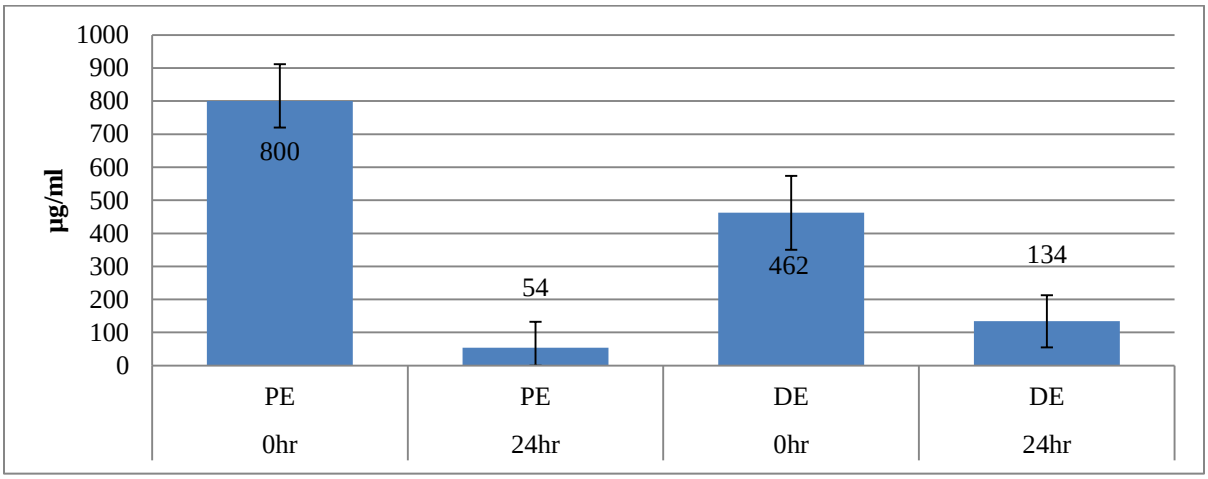



**Figure 7.** Change in total phenolic concentration levels over time in Experiment 1
PE=1ml PFSP Extract, DE=1ml 50% Dilution of PFSP Extract

Table 7 and Figures 8 and 9 show the effect of temperature at 24hr on anthocyanin and total phenolic levels. Both the NT and HT treatments were significantly lower than the 0hr baseline in both PE and DE but there was no significant difference between the NT and HT treatments for either substrate level.

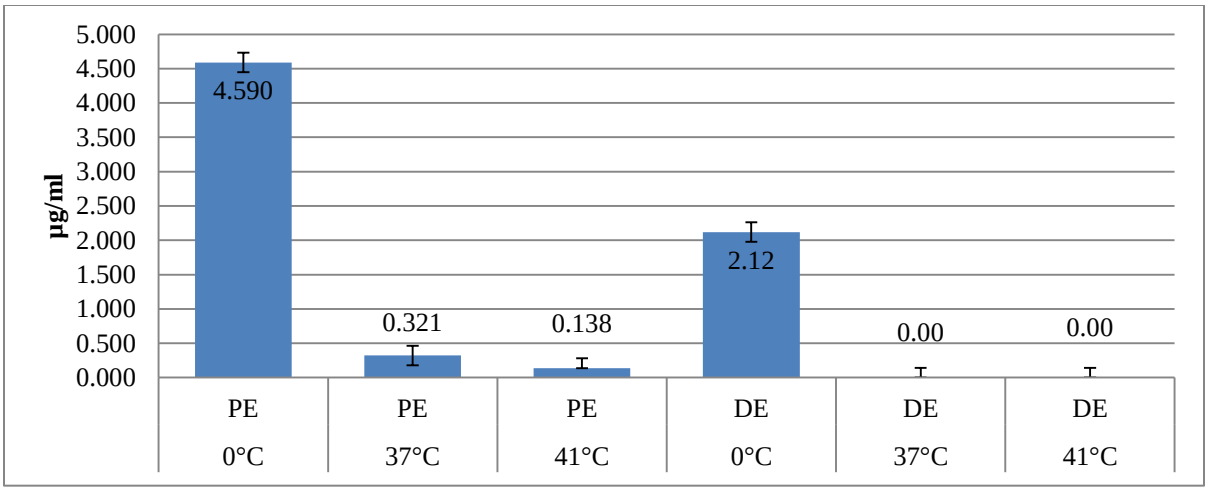



**Figure 8.** Temperature effects on anthocyanin concentrations in Experiment 1
PE=1ml PFSP Extract, DE=1ml 50% Dilution of PFSP Extract

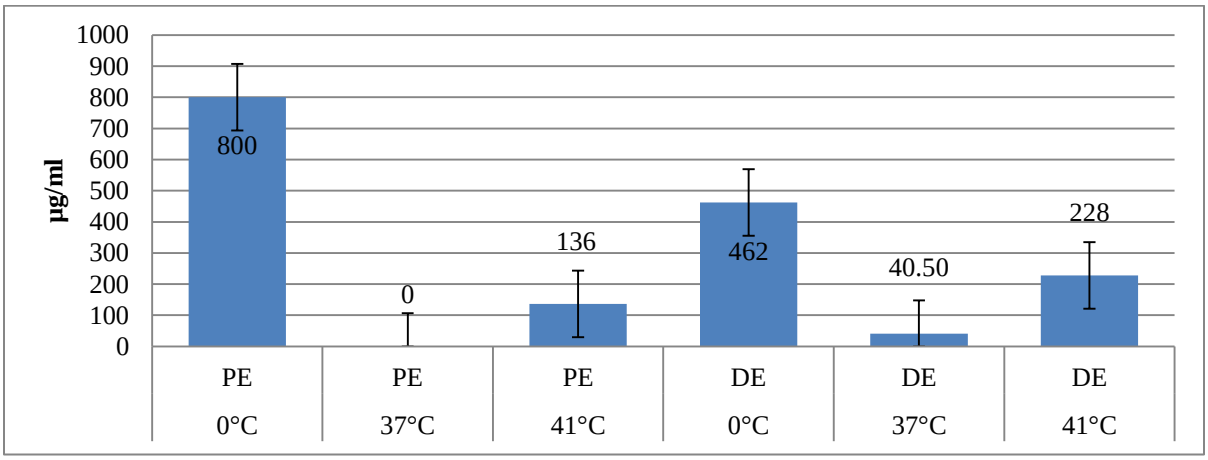



**Figure 9.** Temperature effects on total phenolic concentrations in Experiment 1
PE=1ml PFSP Extract, DE=1ml 50% Dilution of PFSP Extract

*Experiment 2*

After incubation, 10GP bottles were almost completely solid in consistency and were deemed unacceptable for further analysis. 10g of powder was far above the saturation point and any enzymatic activity would have been inhibited. Methane levels for 1GP, 5GP, and blanks at 0hr ranged between 7.68nmol/ml and 19.68nmol/ml. The pH levels at 0hr for

all bottles were between 6.1 and 6.4. Total VFA levels for all bottles at 0hr ranged from 15.9mM to 34.95mM (Table 3).

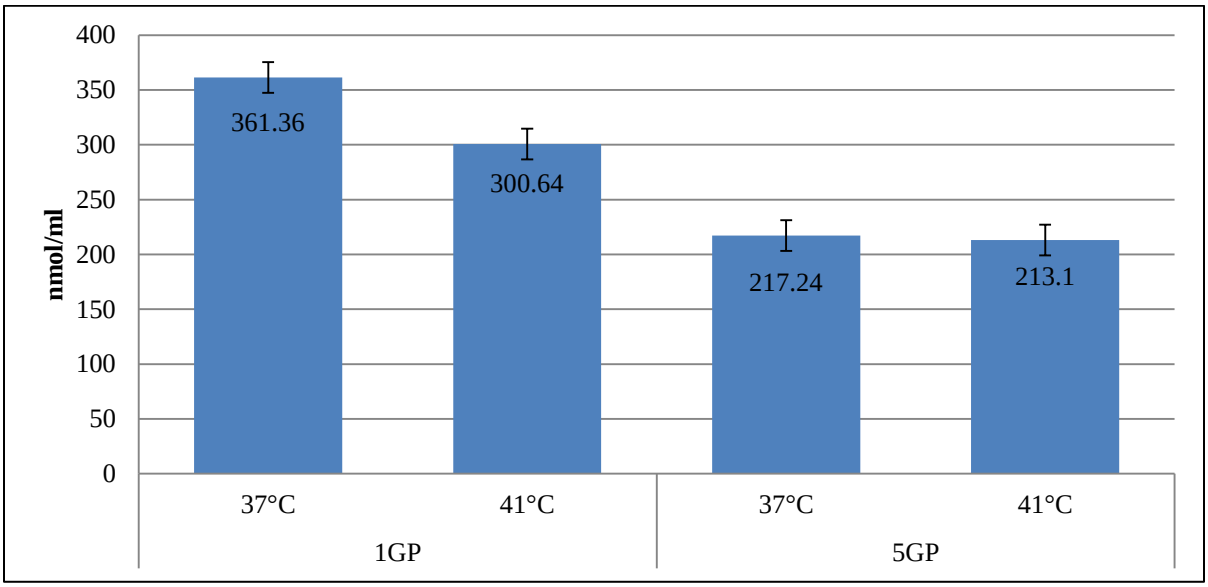
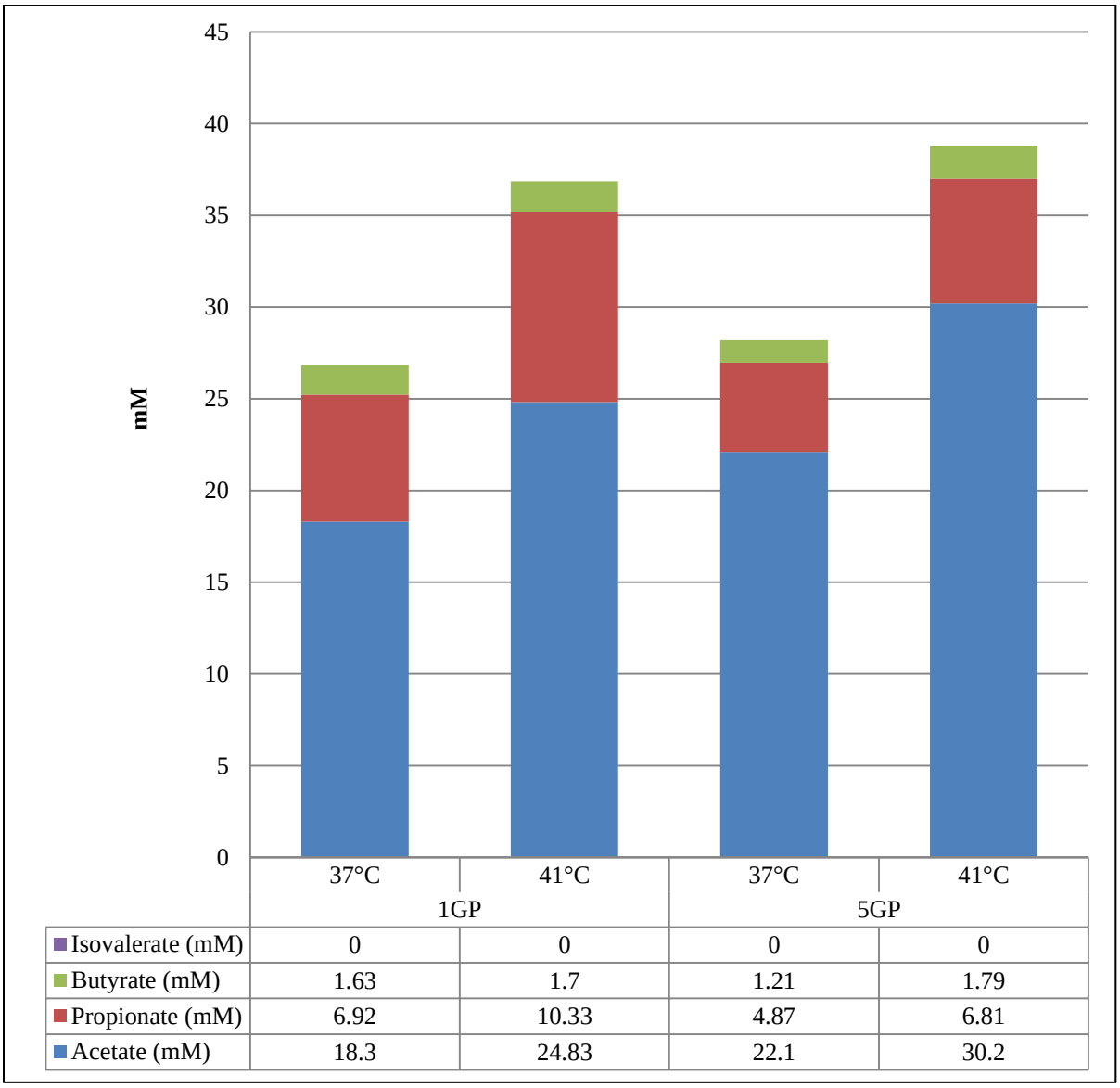



**Figure 10.** Methane concentrations after 12hr incubation in Experiment 2
1GP= 1g freeze dried PFSP powder, 5GP=5g freeze dried PFSP powder

Table 8 shows the values from all 12hr measurements. Blank values were not statistically analyzed and all values have been adjusted by subtracting the blank values from the respective treatments (except for pH). Methane levels for 1GP were 361.4nmol/ml and 300.6nmol/ml for NT and HT, respectively. 5GP methane levels were 217.2nmol/ml and 213.1nmol/ml for NT and HT, respectively (Figure 10). Both treatment and temperature had significant effects on methane levels. The treatment and temperature interaction was trending towards significance. The pH level for all treatments was 4.5.

Temperature had a significant effect on 12hr Total VFA as shown in Table 8 and Figure 11. Total VFA for 1g were 26.85mM for NT and 36.74mM for HT. The 5GP Total

VFA level for NT was 28.32mM and 38.84mM for HT. Individual VFA measurements are also shown in Table 8 and Figure 11. Temperature had a significant effect on both acetate and propionate. Treatment had a significant effect on propionate and a trending effect on acetate.




| | 1GP | | 5GP | |
|---|---|---|---|---|
| Isovalerate (mM) | 0 | 0 | 0 | 0 |
| Butyrate (mM) | 1.63 | 1.7 | 1.21 | 1.79 |
| Propionate (mM) | 6.92 | 10.33 | 4.87 | 6.81 |
| Acetate (mM) | 18.3 | 24.83 | 22.1 | 30.2 |

**Figure 11.** Volatile Fatty Acid concentrations after 12hr incubation in Experiment 2
1GP= 1g freeze dried PFSP powder, 5GP=5g freeze dried PFSP powder

Anthocyanin and total phenolic levels were significantly affected by treatment at 12hrs. The interaction between temperature and treatment was also significant (Table 8).

The 24hr values are listed in Table 9. Methane values were lower for the 5GP treatment level as compared to 1GP. HT treatments trended to have lower levels as well (Figure 12). pH values were significantly higher in HT bottles.

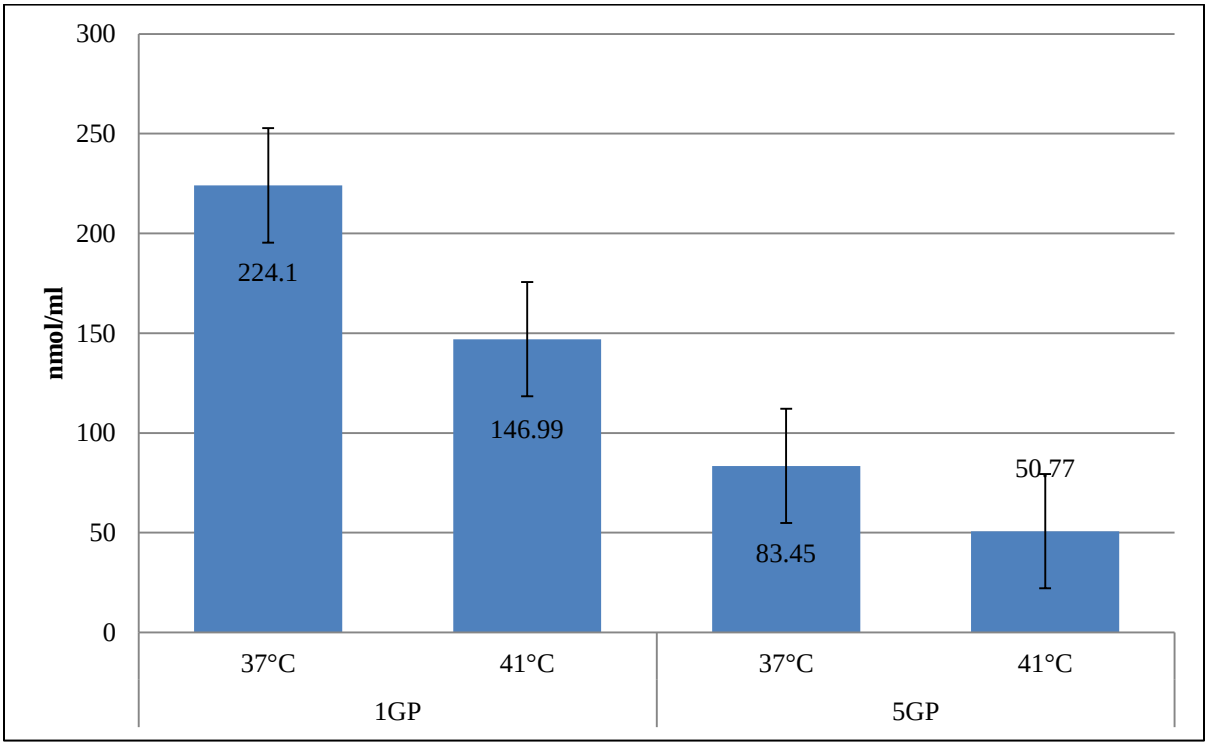
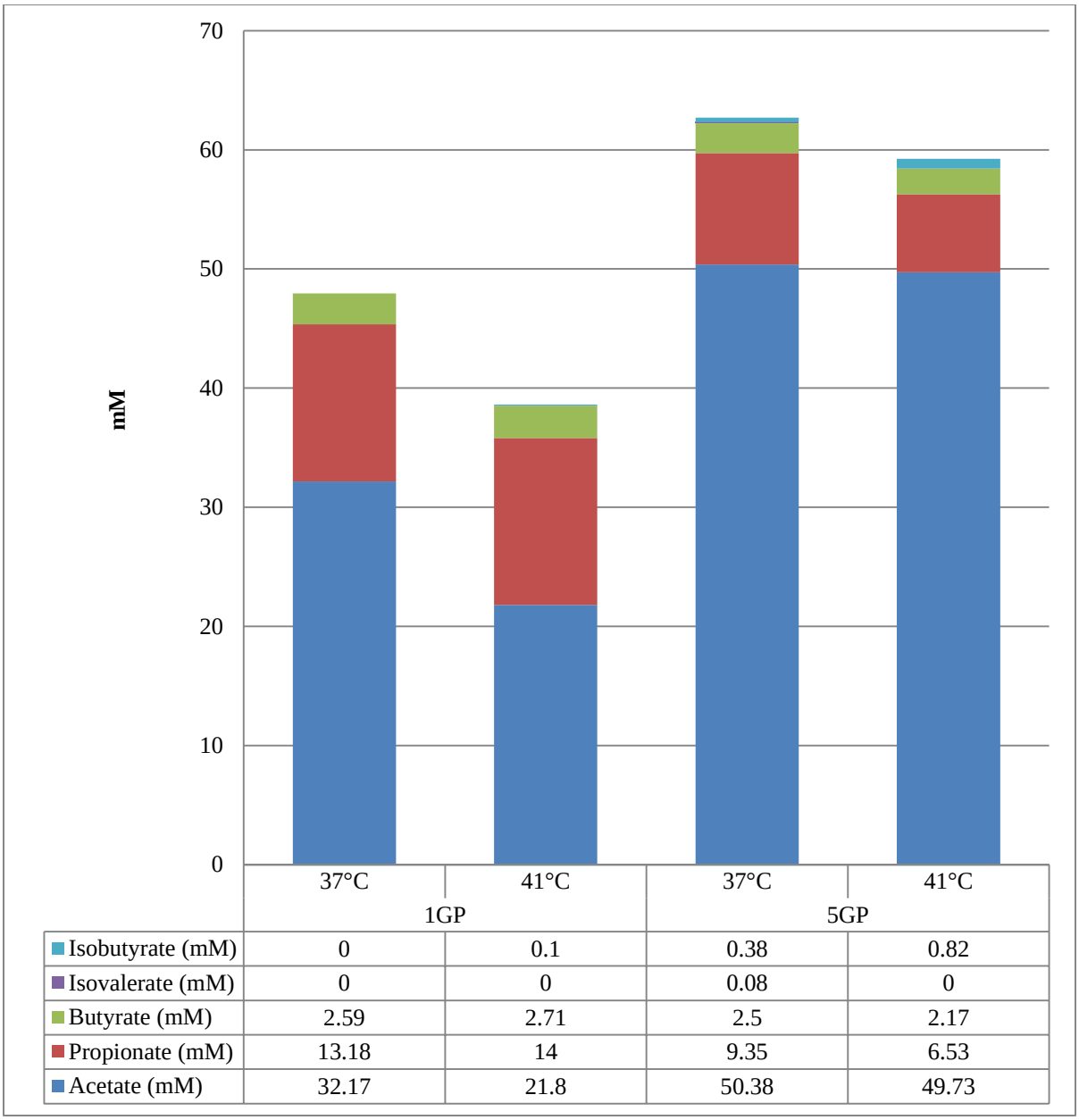



**Figure 12.** Methane concentrations after 24hr incubation in Experiment 2
1GP= 1g freeze dried PFSP powder, 5GP=5g freeze dried PFSP powder

The 24hr Total VFA levels were significantly higher in 5GP treatments and tended to be higher in NT treatments. Acetate levels were significantly higher in 5GP treatments and tended to be higher in NT treatments. In contrast, propionate concentrations were significantly higher in 1GP treatments (Table 5, Figure 13).




| | 37°C (1GP) | 41°C (1GP) | 37°C (5GP) | 41°C (5GP) |
|---|---|---|---|---|
| Isobutyrate (mM) | 0 | 0.1 | 0.38 | 0.82 |
| Isovalerate (mM) | 0 | 0 | 0.08 | 0 |
| Butyrate (mM) | 2.59 | 2.71 | 2.5 | 2.17 |
| Propionate (mM) | 13.18 | 14 | 9.35 | 6.53 |
| Acetate (mM) | 32.17 | 21.8 | 50.38 | 49.73 |

**Figure 13.** Volatile Fatty Acid concentrations after 24hr incubation in Experiment 2
1GP= 1g freeze dried PFSP powder, 5GP=5g freeze dried PFSP powder

Table 10 and Figures 14 and 15 show the anthocyanin and total phenolic levels over time. Treatment effects were significant for both anthocyanin and total phenolics. Time had no significant effect on anthocyanin or total phenolics.

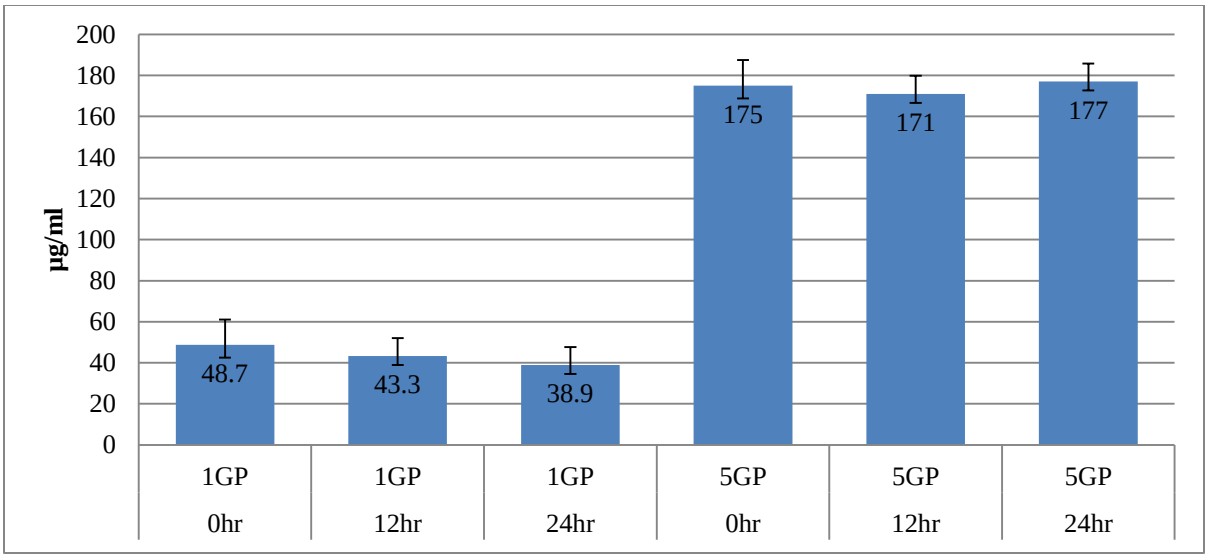



**Figure 14.** Change in anthocyanin concentration over time in Experiment 2
1GP= 1g freeze dried PFSP powder, 5GP=5g freeze dried PFSP powder

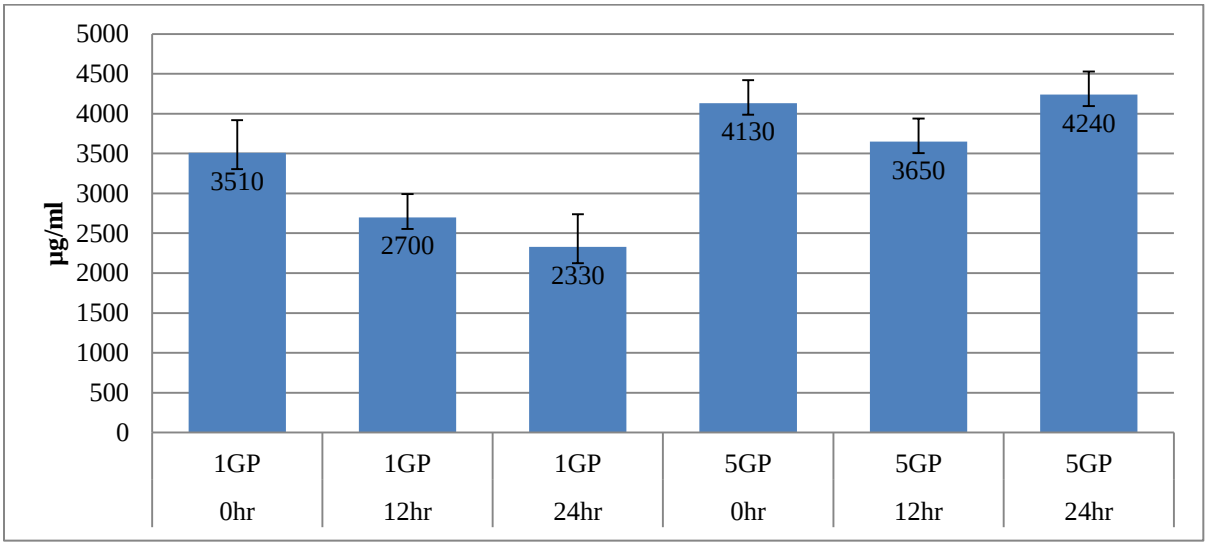



**Figure 15.** Change in total phenolic concentration over time in Experiment 2
1GP= 1g freeze dried PFSP powder, 5GP=5g freeze dried PFSP powder

Table 11 and Figures 16 and 17 show the interaction of anthocyanin and total phenolic levels based on temperature. The 5GP treatments were significantly higher for both anthocyanins and total phenolics. There were no statistical differences between NT and HT.

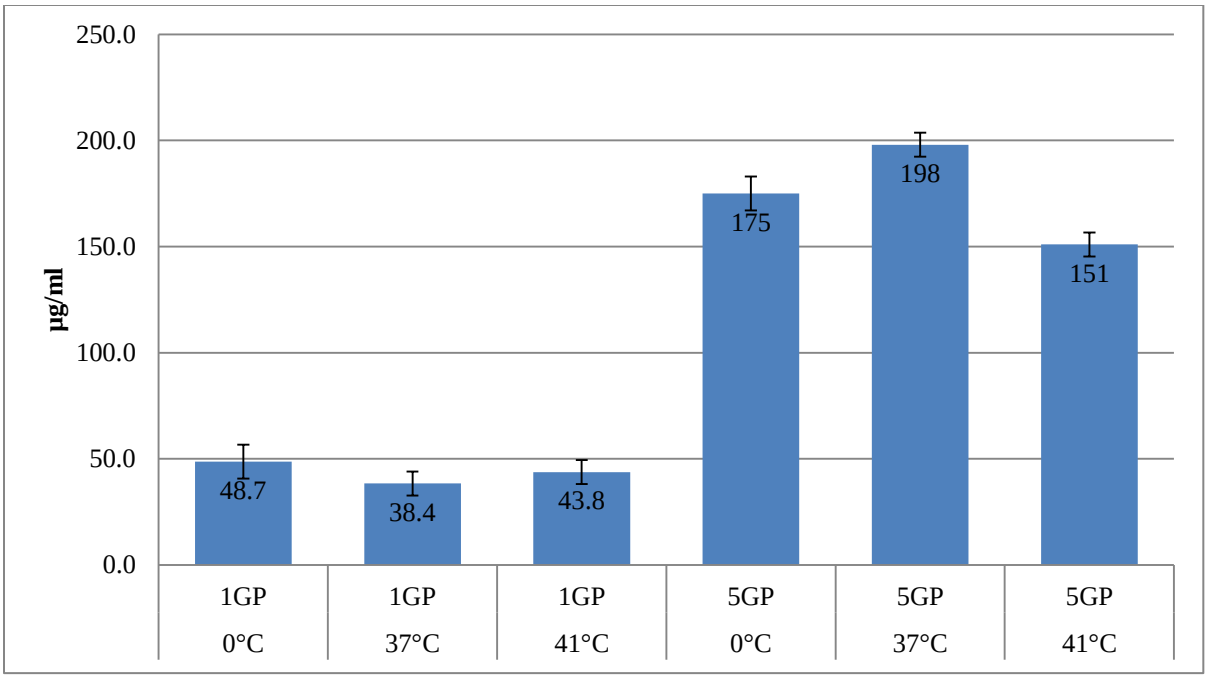



**Figure 16.** Temperature effects on anthocyanin concentration in Experiment 2
1GP= 1g freeze dried PFSP powder, 5GP=5g freeze dried PFSP powder

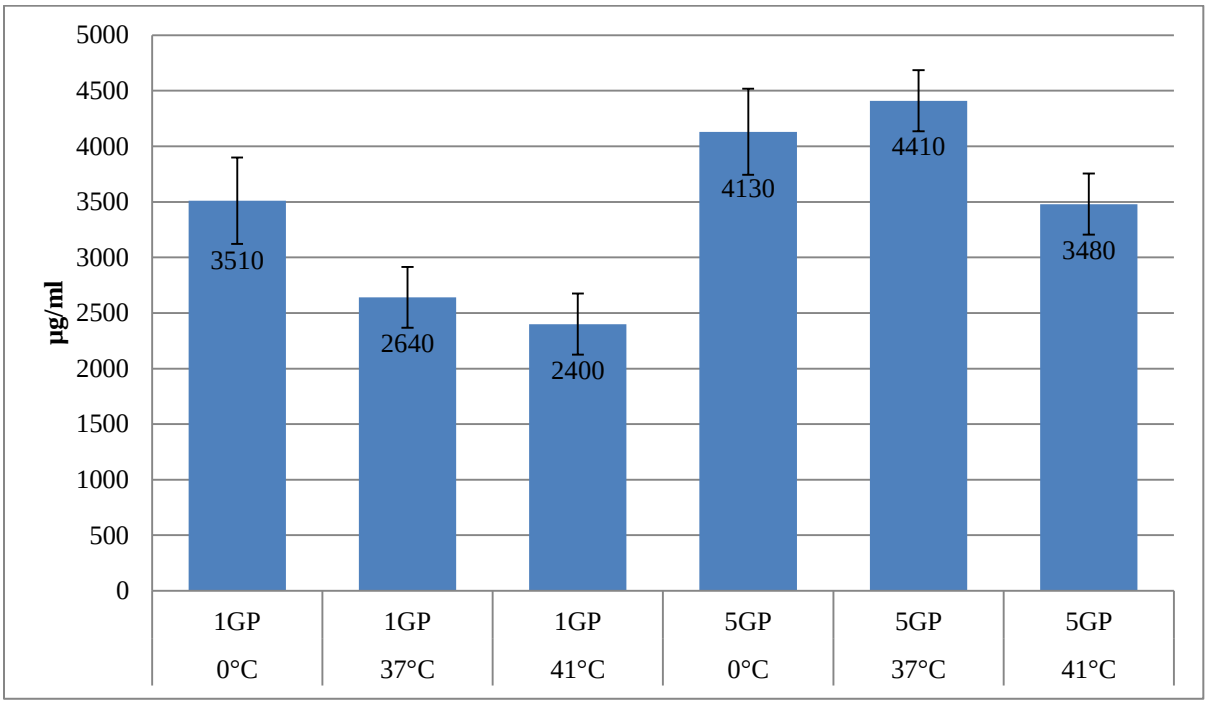



**Figure 17.** Temperature effects on total phenolic concentration in Experiment 2
1GP= 1g freeze dried PFSP powder, 5GP=5g freeze dried PFSP powder

*Experiment 3*

Methane levels for all bottles at 0hr ranged from 5.79nmol/ml to 20.6nmol/ml. All pH values were between 5.7 and 5.8; total VFA concentrations were between 0mM and 46.59mM. All of these 0hr values indicate little to no fermentation, as expected (Table 3). No 0hr treatments were analyzed for significance, except for anthocyanin and total phenolics.

The 24hr methane levels were significantly higher for the Extract (E) and Powder (P) treatments compared to the Control Extract (CE). NT treatments had significantly higher methane than HT treatments (Table 12, Figure 18).

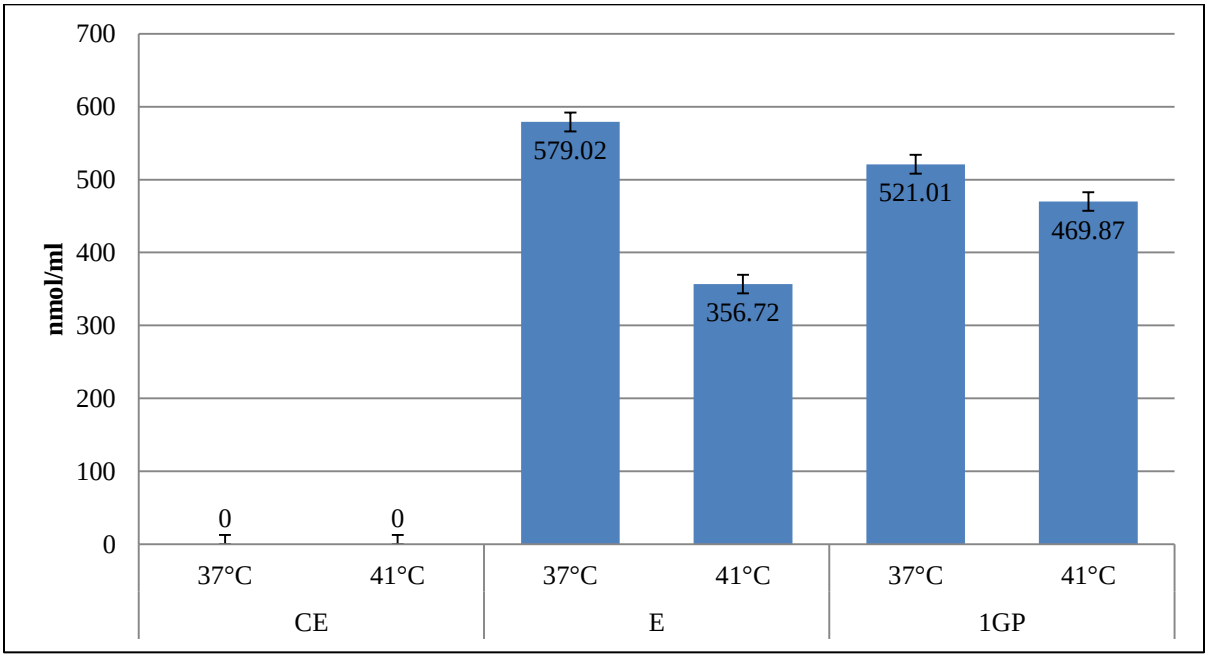
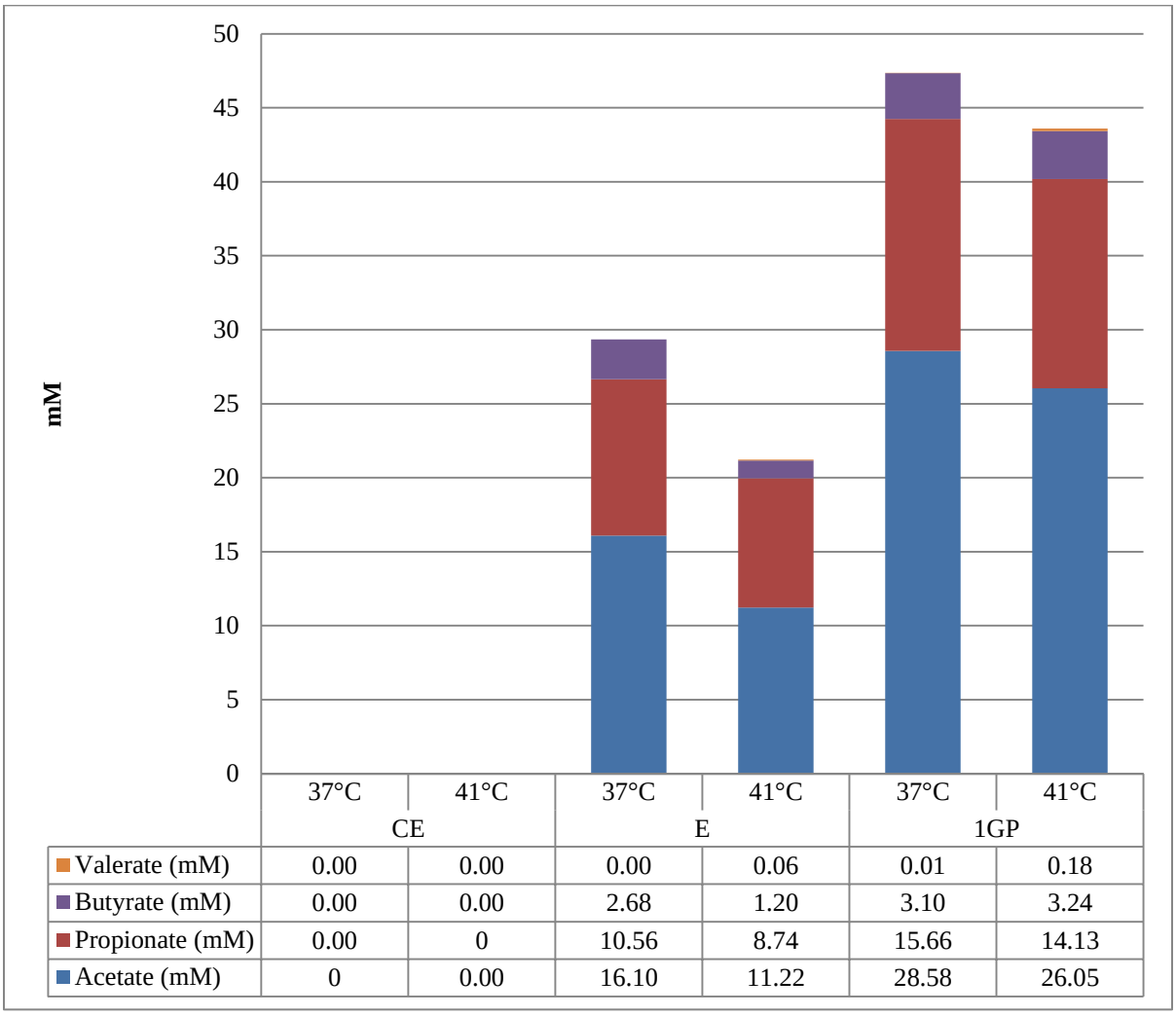



**Figure 18.** Methane concentrations after 24hr incubation in Experiment 3
CE=1.64ml Extract w/o Rumen Fluid, E=1.64ml Extract w/ Rumen Fluid, 1GP=1g Freeze Dried PFSP Powder

The pH of the CE was significantly higher than both the E and P treatments. HT tended to cause a lower pH than NT (Table 12).

No VFA concentrations were detected in the CE. Concentrations were higher with P and tended to be lower with NT. Acetate, propionate, and butyrate were significantly higher in P compared to E (Table 12, Figure 19).




| | CE | | E | | 1GP | |
|---|---|---|---|---|---|---|
| Valerate (mM) | 0.00 | 0.00 | 0.00 | 0.06 | 0.01 | 0.18 |
| Butyrate (mM) | 0.00 | 0.00 | 2.68 | 1.20 | 3.10 | 3.24 |
| Propionate (mM) | 0.00 | 0 | 10.56 | 8.74 | 15.66 | 14.13 |
| Acetate (mM) | 0 | 0.00 | 16.10 | 11.22 | 28.58 | 26.05 |

**Figure 19.** Volatile Fatty Acid concentrations after 24hr incubation in Experiment 3
CE=1.64ml Extract w/o Rumen Fluid, E=1.64ml Extract w/ Rumen Fluid, 1GP=1g Freeze Dried PFSP Powder

Anthocyanin levels were similar for all treatments at 0hr. At 24hr, anthocyanin levels in the CE and P treatments were numerically lower and not statistically significant. The 24hr

anthocyanin levels were significantly lower in the E treatment (Table 13, Figure 20). Similar to anthocyanin concentrations, the total phenolic concentrations were not significantly affected by time in the CE and P treatments. The 24hr Extract treatment had significantly lower total phenolic levels than 0hr (Table 13, Figure 21).

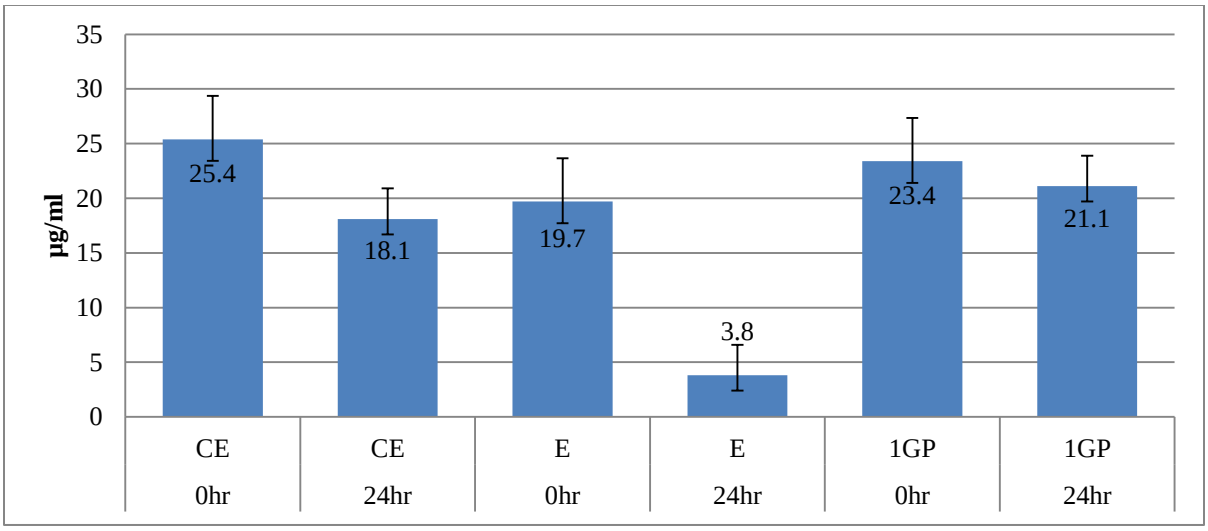



**Figure 20.** Change in anthocyanin concentrations over time in Experiment 3
CE=1.64ml Extract w/o Rumen Fluid, E=1.64ml Extract w/ Rumen Fluid, 1GP=1g Freeze Dried PFSP Powder

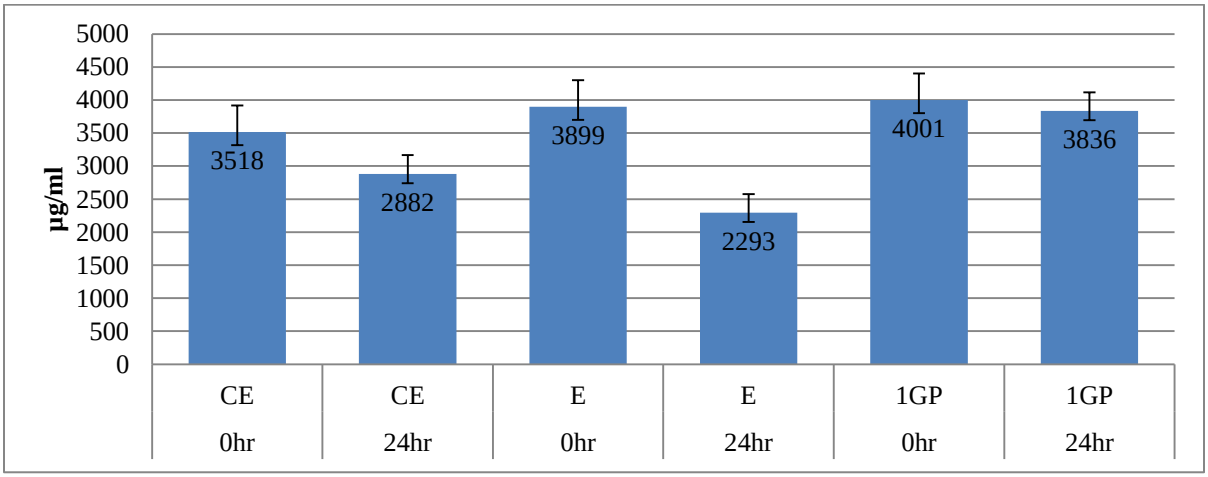



**Figure 21.** Change in total phenolic concentrations over time in Experiment 3
CE=1.64ml Extract w/o Rumen Fluid, E=1.64ml Extract w/ Rumen Fluid, 1GP=1g Freeze Dried PFSP Powder

The HT treatment caused significant reductions in anthocyanin concentrations of the E treatment. The CE and P treatments had lower anthocyanin concentrations at HT but were insignificant (Table 14, Figure 22). Total phenolic concentrations followed a similar pattern. The HT treatment caused a significant reduction in the CE and E versus the 0hr treatment. The NT treatment of E was also lower than the baseline at 0hr (Table 14, Figure 23).

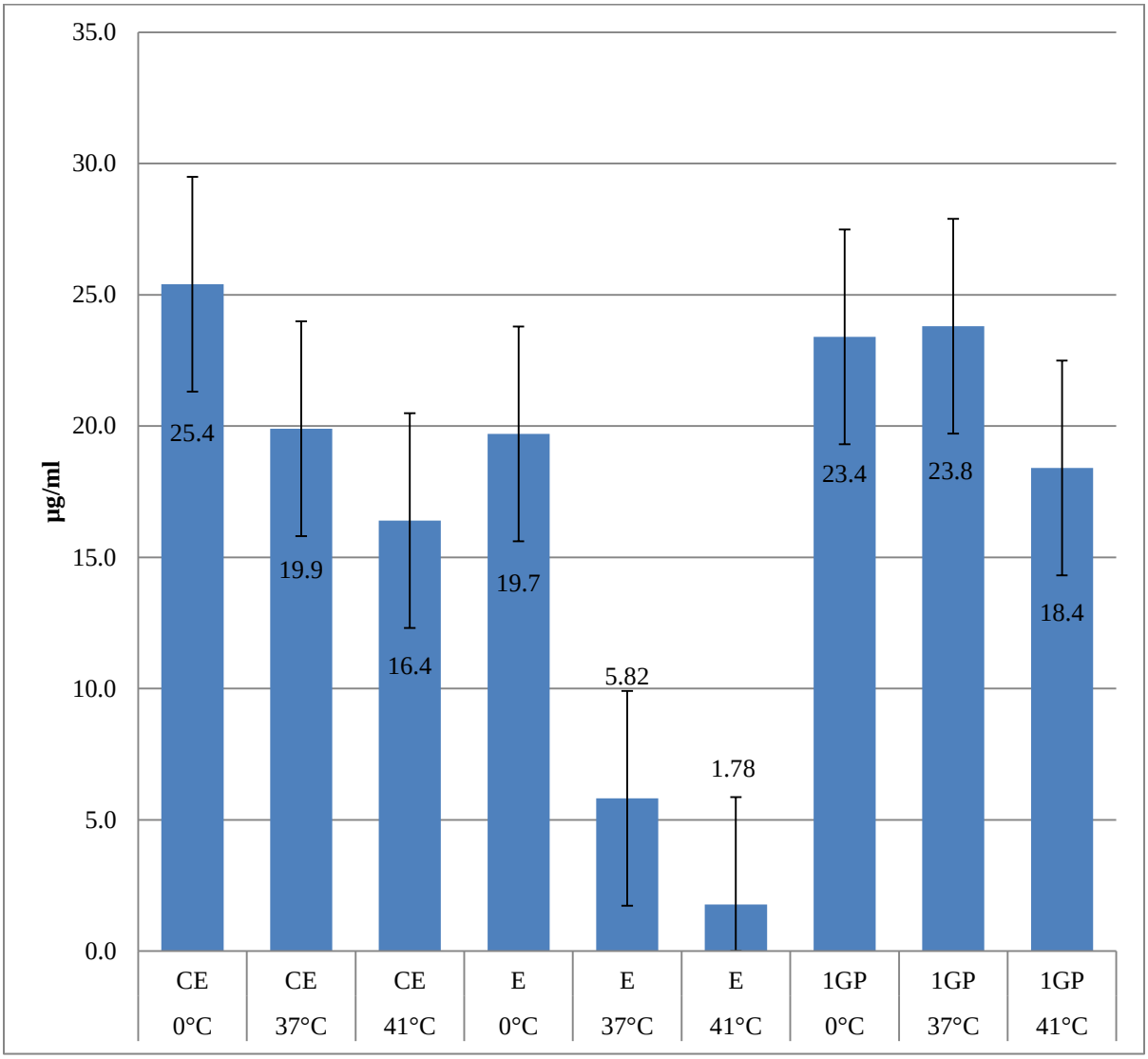



**Figure 22.** Temperature effects on anthocyanin concentrations in Experiment 3
CE=1.64ml Extract w/o Rumen Fluid, E=1.64ml Extract w/ Rumen Fluid, 1GP=1g Freeze Dried PFSP Powder

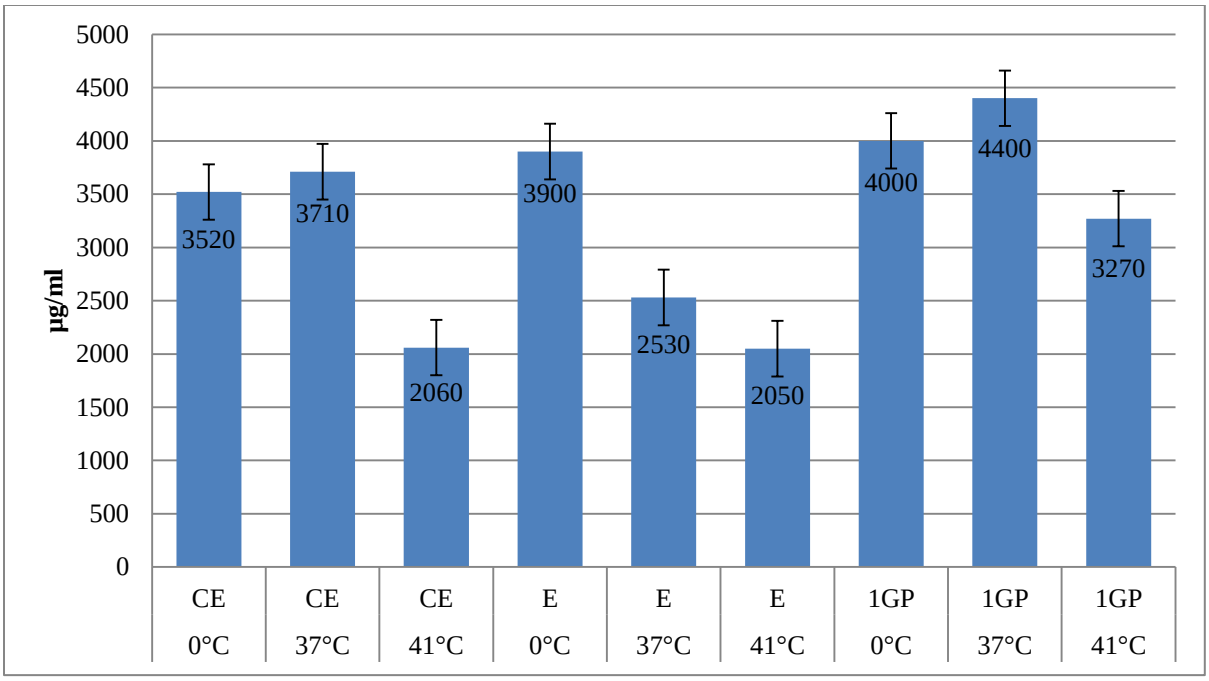



**Figure 23.** Temperature effects on total phenolic concentrations in Experiment 3
CE=1.64ml Extract w/o Rumen Fluid, E=1.64ml Extract w/ Rumen Fluid, 1GP=1g Freeze Dried PFSP Powder

## Discussion

Experiment 1 was conducted largely as a preliminary study in order to assess a potential baseline on which other studies could be conducted. While the temperature of the bovine rumen normally remains a constant 39°C, regardless of ambient or body temperatures (Citation Needed), two incubation temperatures, 37°C (NT) and 41°C (HT) were used in order to simulate possible temperatures of a cow under normal and heat stressed conditions. Anthocyanin-rich extract from PFSP was chosen as the substrate primarily because the anthocyanin concentration can readily be quantified by simple spectrometric readings. Also, because of the ease of quantification of anthocyanin concentration, the extract could easily be made to a standardized level and used as a feed additive.

Experiment 1 showed significant reductions in anthocyanin concentration, regardless of treatment and temperature. These are contrary to the findings of Hosoda et al. (2009), where rumen fermentation appeared to have no effect on the anthocyanin concentration of ensiled anthocyanin-rich corn. After the analyses of Experiment 1, the significant reductions in the anthocyanin concentrations after the 24hr incubation period were originally thought to be caused by the increased pH and temperature, as anthocyanins have been reported to be most stable at a pH around 4 and may remain stable for short periods of time at increased temperatures (Castaneda-Ovando et al., 2009; Eiro & Heinonen, 2002). However, the results of Experiment 3 disprove this assumption and suggest that the reduction in anthocyanin concentration was primarily due to rumen fermentation. This conclusion is supported by the fact that there were no significant reductions in anthocyanins in the CE which was free from rumen fluid and exposed to a higher pH than the E group. If the higher pH and temperatures were truly the only causes of the reduction, then significant reductions should have been evident in both of these groups.

The sensitivity of E to rumen activity could possibly be explained by the processes that occur during the extraction procedure. The extract is predominately composed of water, anthocyanins and other phenolic compounds as most of the starches, fiber, and other components that raw PFSP possess are removed during the extraction of anthocyanins (Steed & Truong, 2008). With these components removed, the anthocyanins are more accessible to rumen microorganisms. This conclusion is furthered emphasized by the results of Experiments 2 and 3 where the anthocyanin content of 1GP and 5GP were not statistically affected by inoculation with rumen fluid. Research also reports that anthocyanins become

more unstable when isolated as well as when not acylated (Bassa & Francis, 1987). While the majority of anthocyanins from PFSP are acylated, the extraction process causes the anthocyanins to become more isolated and thus more susceptible to degradation. This offers a possible explanation as to the higher vulnerability of the extract than the powder.

An interesting phenomenon present in Experiment 2 is the acetate to propionate ratio and its relationship to methane production. Decreased methane production is common with increased feed intake but it is also common with a decrease in the acetate to propionate ratio (Russell, 1998). These data indicate an increase in the acetate to propionate ratio with a decrease in methane production. This is seen between treatments, times, and temperatures. However, Experiment 3 data shows that a decrease in acetate to propionate ratio coincides with a decrease in methane for the E group and for the P group, the only difference is the temperature- the higher temperature had similar acetate to propionate ratio, but lower methane (Table 8). These results indicate that raw PFSP could be a beneficial and efficient feed and would be a better choice, both nutritionally and environmentally, compared to E.

Sweetpotatoes have been shown to be a partial substitute for corn as a potential alternative energy source for cattle to help reduce feed costs, depending on their availability (Thibodeau et al., 2002). PFSP possess similar nutritional attributes as traditional sweetpotatoes and could be used in the same fashion. This practice would be most feasible in regions where PFSP are grown in larger quantities with a high availability of culls and byproducts such as certain Asian countries. However, there is growing interest in PFSP production in the United States because of their beneficial health properties and potential use

as natural colorants (Truong, et al., 2010). The results of this study further strengthen the potential benefits for PFSP to be used for an alternative feed source for dairy cattle.

In summation, the effects that rumen fermentation has on the anthocyanin content of PFSP depend largely on the form in which they are presented in the rumen. These data imply that it is possible that feeding raw PFSP could serve as a possible source of antioxidant to help negate the effects brought on by heat stress in dairy cattle. However, this is largely dependent on whether or not the anthocyanins would be absorbed into the body of dairy cattle from the abomasum and gut. More research is needed to assess the real-life impacts that this could have, including the behavior of anthocyanins in the abomasum and gut of cattle as well as the nutritional effects that PFSP could have on dairy cattle health.

## Literature Cited


Bassa, I., & Francis, F. (1987). Stability of anthocyanins from sweet potatoes in a model beverage. A research note. *Journal of Food Science*, 1753-1754.

Beede, D., & Collier, R. (1986). Potential nutritional strategies for intensively managed cattle during thermal stress. *Journal of Animal Science*, 543-554.

Berman, A., Folman, M., Kaim, M., Mamen, M., Herz, Z., Wolfenson, D., . . . Graber, Y. (1985). Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical climate. *Journal of Dairy Science*, 1488-1495.

Castaneda-Ovando, A., Pacheco-Hernandez, M., Paez-Hernandez, M., Rodriguez, J., & Galan-Vidal, G. (2009). Chemical studies of anthocyanins: a review. *Food Chemistry*, 859-871.

Dijsktra, J., Forbes, J., & France, J. (2005). Introduction. In J. Dijsktra, J. Forbes, & J. France, *Quantitative aspects of ruminant digestion and metabolism* (pp. 1-10). Wallingford, UK: CABI Publishing.

Eiro, M., & Heinonen, M. (2002). Anthocyanin color behavior and stability during storage: effect of intermolecular copigmentation. *Journal of Agricultural and Food Chemistry*, 7461-7466.

Giusti, M., & Wrolstad, R. (2001). Characterization and measurement of anthocyanins by UV-Visible Spectroscopy. In *Current Protocols in Food Analytical Chemistry* (pp. F1.1.1-F.1.2.13). John Wiley & Sons, Inc.

Hosoda, K., Eruden, B., Matsuyama, H., & Shioya, S. (2009). Silage fermenative quality and characteristics of anthocyanin stability of anthocyanin-rich corn (Zea mays L.). *The Asian-Australasian Journal of Animal Science*, 528-533.

Lykkesfeldt, J., & Svendsen, O. (2007). Oxidants and antioxidants in disease: oxidative stress in farm animals . *The Veterinary Journal*, 502-511.

Mazza, G., Kay, C., Cottrell, T., & Holub, B. (2002). Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *Journal of Agriculture and Food Chemistry*, 7731-7737.

Miyazawa, T., Nakagawa, K., Kudo, M., Muraishi, K., & Someya, K. (1999). Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. *Journal of Agriculture and Food Chemistry*, 1083-1091.

Olson, S., & Seidel, Jr, G. (2000). Culture of in vitro-produced bovine embryos with vitamin E improves development development in vitro and after transfer to recipients. *Biology of Reproduction*, 248-252.

Passamonti, S., Vrhovsek, U., Vanzo, A., & Mattivi, F. (2003). The stomach as a site for anthocyanins absorption from food. *Federation of European Biochemical Societies Letters*, 210-213.

Russell, J. (1998). The Importance of pH in the Regulation. *Journal of Dairy Science*, 3222-3230.

Sakatani, M., Suda, I., Oki, T., Kobayashi, S.-i., Kobayashi, S., & Takahashi, M. (2007). Effects of Purple-Fleshed Sweetpotato anthocyanins on development and intracellular redox status of bovine preimplantation embryos exposed to heat shock. *Journal of Reproduction and Development*, 605-614.

Singleton, V., Orthofer, R., & Lamuela-Raventos, R. (1999). Analysis of total phenol and other oxidation substrates and antioxidants by means of Folin-ciocalteau reagent. *Methods in Enzymology*, 152-178.

Steed, L., & Truong, V.-D. (2008). Anthocyanin content, antioxidant activity, and selected physical properties of flowable purple-fleshed sweetpotato purees. *Journal of Food Science*, 215-221.

Thibodeau, M., Poore, M., & Rogers, G. (2002). Health and production aspects of feeding sweetpotato to cattle. *Veterinary Clinics of North America: Food Animal Practice*, 349-365.

Truong, V.-D., Deighton, N., Thompson, R., McFeeters, R., Dean, L., Pecota, K., & Yencho, C. (2010). Characterization of anthocyanins and anthocyanidins in purple-fleshed sweetpotatoes by HPLC-DAD/ESI-MS/MS. *Journal of Agricultural and Food Chemistry*, 404-410.

# APPENDIX

**Table 2.** Total Batch Data for Experiment 1

| | 0hr | | | 24hr | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 37°C | | | 41°C | | |
| | PE | DE | BLANK | PE | DE | BLANK | PE | DE | BLANK |
| Methane (nmol/ml) | 17.28 | 20.01 | 19.19 | 968.5 | 684.3 | 255.9 | 1125 | 1034 | 355.2 |
| pH | 6.3 | 6.2 | 6.2 | 5.9 | 6.1 | 6.0 | 6.0 | 6.1 | 5.9 |
| Anthocyanin (µg/mL) | 4.93 | 2.46 | 0.342 | 0.321 | 0 | 0 | 0.138 | 0 | 0 |
| Total Phenolics (µg/mL) | 3104 | 2766 | 2304 | 2232 | 2299 | 2259 | 2503 | 2594 | 2367 |
| Acetate (mM) | 17.0 | 17.2 | 14.9 | 26.1 | 22.6 | 17.9 | 24.6 | 22.8 | 19.8 |
| Propionate (mM) | 3.50 | 3.56 | 3.20 | 9.67 | 6.35 | 3.98 | 8.89 | 6.28 | 4.52 |
| Isobutyrate (mM) | . | . | . | . | . | . | . | . | . |
| Butyrate (mM) | 2.65 | 2.65 | 2.59 | 5.04 | 4.02 | 3.20 | 4.50 | 3.95 | 3.59 |
| Isovalerate (mM) | 0.0725 | 0.0625 | 0.0750 | 0.220 | 0.298 | 0.300 | 0.328 | 0.365 | 0.450 |
| Valerate (mM) | . | . | . | 0.0750 | . | . | 0.0100 | . | . |
| VFA Total (mM) | 23.2 | 23.5 | 20.8 | 41.0 | 33.3 | 25.4 | 38.3 | 33.4 | 28.3 |

Values are derived from averages of two repetitions
PE= 1ml Purple Sweet Potato Extract, DE= 1ml 50% Diluted Purple Sweet Potato Extract, BLANK= Rumen fluid & buffer without substrate

**Table 3.** Total Batch Date for Experiment 2

| | 0hr | | | 12hr | | | | | | 24hr | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 37°C | | | 41°C | | | 37°C | | | 41°C | | |
| | 1GP | 5GP | BLANK | 1GP | 5GP | BLANK | 1GP | 5GP | BLANK | 1GP | 5GP | BLANK | 1GP | 5GP | BLANK |
| Methane (nmol/ml) | 14.34 | 9.130 | 17.99 | 512.5 | 368.4 | 151.2 | 516.7 | 429.2 | 216.1 | 389.7 | 249.0 | 165.6 | 404.7 | 308.5 | 257.7 |
| pH | 6.3 | 6.2 | 6.4 | 4.5 | 4.5 | 6.5 | 4.5 | 4.5 | 6.4 | 4.4 | 4.4 | 6.7 | 4.5 | 4.5 | 6.6 |
| Anthocyanin (µg/mL) | 48.7 | 175 | 0 | 38.9 | 188 | 0 | 47.9 | 154 | 0.234 | 37.8 | 207 | 0 | 39.9 | 147 | 0 |
| Total Phenolics (µg/mL) | 6071 | 6685 | 2561 | 5240 | 6670 | 2675 | 5653 | 6116 | 2819 | 5181 | 7299 | 2480 | 5002 | 6710 | 3043 |
| Acetate (mM) | 16.5 | 26.2 | 20.2 | 38.2 | 42.0 | 19.9 | 44.0 | 49.3 | 19.1 | 41.9 | 60.1 | 9.74 | 48.3 | 76.3 | 26.6 |
| Propionate (mM) | 0.87 | 1.64 | 1.76 | 8.86 | 6.81 | 1.94 | 12.7 | 9.22 | 2.41 | 14.1 | 10.3 | 0.96 | 17.1 | 9.60 | 3.07 |
| Isobutyrate (mM) | . | . | . | . | 0.148 | . | . | 0.147 | . | . | 0.533 | 0.16 | 0.15 | 0.87 | 0.0475 |
| Butyrate (mM) | 0.553 | 0.702 | 0.765 | 2.53 | 2.10 | . | 2.86 | 2.95 | 1.16 | 2.73 | 2.64 | 0.14 | 3.27 | 2.73 | 0.555 |
| Isovalerate (mM) | . | 0.0200 | . | . | . | . | . | 0.0500 | 0.250 | . | 0.118 | 0.035 | . | 0.125 | 0.223 |
| Valerate (mM) | . | 0.135 | . | . | . | . | . | . | . | . | 0.0400 | . | . | . | . |
| VFA Total (mM) | 18.0 | 28.7 | 22.7 | 49.6 | 51.1 | 22.8 | 59.6 | 61.7 | 22.8 | 58.8 | 73.7 | 10.9 | 68.8 | 89.6 | 30.4 |

Values are derived from averages of three repetitions
1GP= 1g Purple Sweet Potato Powder; 5GP= 5g Purple Sweet Potato Powder; BLANK= Rumen fluid & buffer without substrate

**Table 4.** Total Batch Data for Experiment 3

| | 0hr | | | | 24hr | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 37℃ | | | | 41℃ | | | |
| | 1GP | E | CE | BLANK | 1GP | E | CE | BLANK | 1GP | E | CE | BLANK |
| Methane (nmol/ml) | 17.07 | 8.487 | 10.08 | 11.88 | 731.1 | 789.1 | 14.26 | 210.1 | 757.1 | 644.0 | 11.66 | 287.2 |
| pH | 5.8 | 5.7 | 5.8 | 5.8 | 4.2 | 4.6 | 6.0 | 6.0 | 4.1 | 4.9 | 6.3 | 6.4 |
| Anthocyanin (µg/mL) | 25.3 | 21.6 | 27.3 | 1.89 | 24.7 | 6.74 | 20.8 | 0.927 | 22.6 | 5.94 | 20.5 | 4.17 |
| Total Phenolics (µg/mL) | 5750 | 5647 | 5266 | 1748 | 6357 | 4482 | 5657 | 1950 | 6642 | 5430 | 5434 | 3377 |
| Acetate (mM) | 30.1 | 21.8 | 0 | 22.8 | 53.1 | 40.6 | 0 | 24.5 | 54.7 | 39.9 | 0 | 28.7 |
| Propionate (mM) | 6.23 | 6.11 | 0 | 5.04 | 19.9 | 14.8 | 0 | 4.20 | 19.47 | 14.1 | 0 | 5.34 |
| Isobutyrate (mM) | 0.243 | 0.393 | 0 | 0.215 | 0.247 | 0.143 | 0 | 0.250 | 0.257 | 0.183 | 0 | 0.305 |
| Butyrate (mM) | 2.36 | 1.93 | 0 | 1.91 | 5.27 | 4.85 | 0 | 2.17 | 6.30 | 4.26 | 0 | 3.06 |
| Isovalerate (mM) | 0.423 | 0.333 | 0 | 0.425 | 0.400 | 0.313 | 0 | 0.740 | 0.420 | 0.367 | 0 | 0.655 |
| Valerate (mM) | 0.227 | 0.0700 | 0 | 0.0150 | 0.260 | 0.110 | 0.0500 | 0.250 | 0.233 | 0.107 | 0 | 0.0500 |
| VFA Total (mM) | 39.6 | 30.7 | 0 | 30.4 | 79.1 | 60.8 | 0.05 | 32.1 | 81.4 | 58.9 | 0 | 38.1 |

Values are derived from averages of three repetitions
CE= 1.64mL Control Extract; E= 1.64mL Extract; 1GP= 1g Purple Sweet Potato Powder; BLANK= Rumen fluid & buffer without substrate

**Table 5.** Effect of purple-fleshed sweetpotato extract on methane, pH, volatile fatty acids, anthocyanin, and total phenolic concentrations after 24hr incubation with rumen fluid culture (Experiment 1)

| | PE | | DE | | SE | P value | | |
|---|---|---|---|---|---|---|---|---|
| | 37°C | 41°C | 37°C | 41°C | | trt | temp | trt*temp |
| Methane (nmol/ml) | 712.6$^{ab}$ | 769.7$^{a}$ | 428.3$^{b}$ | 678.5$^{ab}$ | 85.57 | 0.0933 | 0.1469 | 0.3225 |
| pH | 5.9 | 6.0 | 6.1 | 6.1 | 0.0661 | 0.1318 | 0.3202 | 0.3202 |
| Anthocyanin (µg/mL) | 0.321 | 0.138 | 0 | 0 | 0.089 | 0.0616 | 0.3608 | 0.3608 |
| Phenolics (µg/mL) | 0 | 136 | 41.0 | 228 | 119.1 | 0.5385 | 0.2156 | 0.9246 |
| Acetate (mM) | 8.14 | 4.81 | 4.67 | 3.02 | 1.655 | 0.1868 | 0.2072 | 0.6394 |
| Propionate (mM) | 5.70$^{a}$ | 4.38$^{ab}$ | 2.37$^{ab}$ | 1.76$^{b}$ | 1.003 | 0.0415 | 0.3905 | 0.7413 |
| Butyrate (mM) | 1.85 | 0.910 | 0.820 | 0.360 | 0.0285 | 0.1661 | 0.2085 | 0.6369 |
| Isovalerate (mM) | - | - | - | - | 0.0285 | 0.1259 | 0.0848 | 0.5216 |
| Total VFA (mM) | 15.6 | 9.98 | 7.85 | 5.05 | 3.085 | 0.1086 | 0.2425 | 0.6676 |

Superscripts denote statistically significant differences, $p < 0.05$
PE= 1ml Purple-fleshed Sweetpotato Extract, DE= 1ml 50% Diluted Purple-fleshed Sweetpotato Extract

**Table 6.** Changes in anthocyanin and total phenolic concentrations of purple-fleshed sweetpotato extract in rumen fluid culture after 24 hours of incubation (Experiment 1)

| | PE | | DE | | P < | | |
|---|---|---|---|---|---|---|---|
| | **0hr** | **24hr** | **0hr** | **24hr** | **trt** | **time** | **trt*time** |
| Anthocyanin (µg/ml) | 4.59[a] | 0.229[b] | 2.12[c] | 0[c] | <0.0001 | <0.0001 | <0.0001 |
| Phenolics (µg/ml) | 800[a] | 54.0[b] | 462[ac] | 134[bd] | 0.2178 | 0.0005 | 0.0621 |

Superscripts denote statistically significant differences, $p < 0.05$

PE= 1ml Purple-Fleshed Sweetpotato Extract, DE= 1ml 50% Diluted Purple-Fleshed Sweetpotato Extract

**Table 7.** Effect of temperature on anthocyanin and total phenolic concentrations of rumen fluid culture with Purple-Fleshed Sweetpotato extract after 24 hours of incubation (Experiment 1)

| | PE | | | DE | | | P < | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0hr** | **37°C** | **41°C** | **0hr** | **37°C** | **41°C** | **trt** | **temp** | **trt*temp** |
| Anthocyanin (µg/mL) | 4.59[a] | 0.321[bd] | 0.138[bd] | 2.12[c] | 0[d] | 0[d] | 0.0002 | <0.0001 | 0.0003 |
| Phenolics (µg/mL) | 800[a] | 0[b] | 136[bc] | 462[ac] | 40.5[b] | 228[bc] | 0.5216 | 0.0028 | 0.1572 |

Superscripts denote statistically significant differences, $p < 0.05$

PE= 1ml Purple-Fleshed Sweetpotato Extract, DE= 1ml 50% Diluted Purple-Fleshed Sweetpotato Extract

**Table 8.** Effect of Purple-Fleshed Sweetpotato powder on methane, pH, volatile fatty acids, anthocyanin, and total phenolic concentrations after 12hr incubation with rumen fluid culture (Experiment 2)

| | 1GP | | 5GP | | SE | P value | | |
|---|---|---|---|---|---|---|---|---|
| | 37°C | 41°C | 37°C | 41°C | | trt | temp | trt*temp |
| Methane (nmol/ml) | 361.4[a] | 300.6[b] | 217.2[c] | 213.1[c] | 14.049 | <0.0001 | 0.0498 | 0.0789 |
| pH | 4.5 | 4.5 | 4.5 | 4.5 | 0 | - | - | - |
| Anthocyanin (μg/mL) | 38.9[a] | 47.6[a] | 188[b] | 154[c] | 8.72 | <0.0001 | 0.1997 | 0.0456 |
| Phenolics (μg/mL) | 2570[a] | 2830[ac] | 4000[b] | 3300[c] | 162.9 | 0.0004 | 0.2138 | 0.018 |
| Acetate (mM) | 18.3[a] | 24.8[ab] | 22.1[a] | 30.2[b] | 2.2 | 0.0703 | 0.0103 | 0.7311 |
| Propionate (mM) | 6.92[a] | 10.3[b] | 4.87[c] | 6.81[a] | 0.421 | 0.0002 | 0.0002 | 0.1189 |
| Butyrate (mM) | 1.63 | 1.70 | 1.21 | 1.79 | 0.221 | 0.4631 | 0.1809 | 0.2782 |
| Isovalerate (mM) | - | - | - | - | - | - | - | - |
| Total VFA (mM) | 26.9[a] | 36.7[b] | 28.3[a] | 38.8[b] | 2.542 | 0.5024 | 0.0039 | 0.9044 |

Superscripts denote statistically significant differences, $p < 0.05$
1GP= 1g Purple-Fleshed Sweetpotato Powder; 5GP= 5g Purple-Fleshed Sweetpotato Powder

**Table 9.** Effect of Purple-Fleshed Sweetpotato powder on methane, pH, volatile fatty acids, anthocyanin, and total phenolic concentrations after 24hr incubation with rumen fluid culture (Experiment 2)

| | 1GP | | 5GP | | SE | P value | | |
|---|---|---|---|---|---|---|---|---|
| | 37°C | 41°C | 37°C | 41°C | | trt | temp | trt*temp |
| Methane (nmol/ml) | $224.1^{a}$ | $147.0^{ab}$ | $83.45^{bc}$ | $50.77^{c}$ | 28.64 | 0.0033 | 0.0916 | 0.4602 |
| pH | $4.4^{a}$ | $4.5^{b}$ | $4.4^{a}$ | $4.5^{ab}$ | 0.008726 | <0.0001 | 0.0109 | 0.0076 |
| Anthocyanin (µg/mL) | $37.8^{a}$ | $40.0^{a}$ | $207^{b}$ | $147^{c}$ | 565.3 | <0.0001 | 0.0109 | 0.0076 |
| Phenolics (µg/mL) | $2700^{a}$ | $1960^{a}$ | $4820^{b}$ | $3670^{ab}$ | 16,962 | 0.0096 | 0.1337 | 0.7261 |
| Acetate (mM) | $32.2^{a}$ | $21.8^{b}$ | $50.4^{c}$ | $49.7^{c}$ | 2.766 | <0.0001 | 0.0814 | 0.1168 |
| Propionate (mM) | $13.18^{a}$ | $14^{a}$ | $9.35^{b}$ | $6.53^{b}$ | 1.11 | 0.0009 | 0.3963 | 0.1401 |
| Isobutyrate (mM) | - | 0.1 | 0.38 | 0.82 | 0.357 | 0.2558 | 0.4164 | - |
| Butyrate (mM) | 2.59 | 2.71 | 2.5 | 2.17 | 0.278 | 0.287 | 0.7194 | 0.4443 |
| Isovalerate (mM) | - | - | 0.0800 | 0 | 0.0437 | - | 0.1055 | - |
| Total VFA (mM) | $47.9^{a}$ | $38.3^{b}$ | $62.8^{c}$ | $59.1^{c}$ | 3.095 | 0.0004 | 0.0643 | 0.3708 |

Superscripts denote statistically significant differences, $p < 0.05$
1GP= 1g Purple-Fleshed Sweetpotato Powder; 5GP= 5g Purple-Fleshed Sweetpotato Powder

**Table 10.** Changes in anthocyanin and total phenolic concentrations of Purple-Fleshed Sweetpotato powder in rumen fluid culture after 12 and 24 hours of incubation (Experiment 2)

| | 1GP | | | 5GP | | | P < | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0hr** | **12hr** | **24hr** | **0hr** | **12hr** | **24hr** | **trt** | **time** | **trt*time** |
| Anthocyanin (μg/mL) | 48.7[a] | 43.3[a] | 38.9[a] | 175[b] | 171[b] | 177[b] | <0.0001 | 0.915 | 0.7851 |
| Phenolics (μg/mL) | 3510[a] | 2700[ab] | 2330[b] | 4130[a] | 3650[a] | 4240[a] | 0.0003 | 0.2014 | 0.1346 |

Superscripts denote statistically significant differences, $p < 0.05$

1GP= 1g Purple-Fleshed Sweetpotato Powder; 5GP= 5g Purple-Fleshed Sweetpotato Powder

**Table 11.** Effect of temperature on anthocyanin and total phenolic concentrations after of rumen fluid culture with Purple-Fleshed Sweetpotato powder (Experiment 2)

| | 1GP | | | 5GP | | | P < | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0hr** | **37°C** | **41°C** | **0hr** | **37°C** | **41°C** | **trt** | **temp** | **trt*temp** |
| Anthocyanin (μg/mL) | 48.7[a] | 38.4[a] | 43.8[a] | 175[b] | 198[b] | 151[b] | <0.0001 | 0.0045 | 0.0005 |
| Phenolics (μg/mL) | 3510[a] | 2640[a] | 2400[b] | 4130[ac] | 4410[c] | 3480[ac] | 0.0002 | 0.0298 | 0.209 |

Superscripts denote statistically significant differences, $p < 0.05$

1GP= 1g Purple-Fleshed Sweetpotato Powder; 5GP= 5g Purple-Fleshed Sweetpotato Powder

**Table 12.** Effect of Purple-Fleshed Sweetpotato extract and powder on methane, pH, volatile fatty acids, anthocyanin, and total phenolic concentrations after 24hr incubation with rumen fluid culture (Experiment 3)

| | CE | | E | | 1GP | | SE | P Value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 37°C | 41°C | 37°C | 41°C | 37°C | 41°C | | trt | temp | trt*temp |
| Methane (nmol/ml) | - | - | 579.02[a] | 356.72[b] | 521.01[c] | 469.87[d] | 12.831 | <0.0001 | <0.0001 | <0.0001 |
| pH | 5.97[a] | 6.27[b] | 4.63[c] | 4.90[d] | 4.17[e] | 4.10[e] | 0.0593 | <0.0001 | 0.0049 | 0.0169 |
| Anthocyanin (µg/mL) | 19.9[a] | 16.4[a] | 5.82[b] | 1.78[b] | 23.8[a] | 18.4[a] | 2.549 | <0.0001 | 0.0612 | 0.9346 |
| Phenolics (µg/mL) | 3710[ac] | 2060[b] | 2530[b] | 2050[b] | 4410[a] | 3270[c] | 239.3 | 0.0001 | 0.0001 | 0.0871 |
| Acetate (mM) | - | - | 16.1[a] | 11.2[a] | 28.6[b] | 26.1[b] | 1.614 | 0.0001 | 0.0975 | 0.5682 |
| Propionate (mM) | - | - | 10.6[a] | 8.74[a] | 15.7[b] | 14.1[b] | 0.665 | 0.0002 | 0.074 | 0.863 |
| Isobutyrate (mM) | - | - | - | - | - | - | 0.0261 | 0.0244 | 0.3749 | 0.651 |
| Butyrate (mM) | - | - | 2.68[a] | 1.20[b] | 3.10[a] | 3.24[a] | 0.258 | 0.0046 | 0.0685 | 0.0338 |
| Isovalerate (mM) | - | - | - | - | - | - | 0.0269 | 0.0667 | 0.0062 | 0.6271 |
| Valerate (mM) | - | - | - | 0.0570[a] | 0.0100[a] | 0.180[b] | 0.0582 | 0.1085 | 0.0209 | 0.864 |
| Total VFA (mM) | - | - | 28.7[a] | 20.8[a] | 47.0[b] | 43.3[b] | 2.33 | <0.0001 | 0.0604 | 0.4575 |

*Blank values not tested for significance.
CE= 1.64mL Control Extract; E= 1.64mL Extract; 1GP= 1g Purple-Fleshed Sweetpotato Powder

**Table 13.** Changes in anthocyanin and total phenolic concentrations of rumen fluid culture with Purple-Fleshed Sweetpotato powder and extract after 24 hours of incubation (Experiment 3)

| | CE | | E | | 1GP | | P value | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0hr | 24hr | 0hr | 24hr | 0hr | 24hr | trt | time | trt*time |
| Anthocyanin (μg/mL) | 25.4[a] | 18.1[a] | 19.7[a] | 3.8[b] | 23.4[a] | 21.1[a] | 0.0113 | 0.0054 | 0.1394 |
| Phenolics (μg/mL) | 3518[ac] | 2882[ab] | 3899[a] | 2293[b] | 4001[c] | 3836[c] | 0.1634 | 0.0098 | 0.1241 |

Superscripts denote statistically significant differences, $p < 0.05$

CE= 1.64mL Control Extract (extract without rumen fluid); E= 1.64mL Extract (extract with rumen fluid); 1GP= 1g Purple-Fleshed Sweetpotato Powder

**Table 14.** Effect of temperature on anthocyanin and total phenolic concentrations of rumen fluid culture with Purple-Fleshed Sweetpotato powder and extract after 24 hours of incubation (Experiment 3)

| | CE | | | E | | | 1GP | | | P < | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0hr | 37°C | 41°C | 0hr | 37°C | 41°C | 0hr | 37°C | 41°C | trt | temp | trt*temp |
| Anthocyanin (μg/mL) | 25.4[a] | 19.9[a] | 16.4[a] | 19.7[a] | 5.82[b] | 1.78[b] | 23.4[a] | 23.8[a] | 18.4[a] | 0.0021 | 0.0171 | 0.453 |
| Phenolics (μg/mL) | 3520[a] | 3710[a] | 2060[b] | 3900[ac] | 2530[b] | 2050[b] | 4000[ac] | 4400[c] | 3270[a] | 0.003 | <0.0001 | 0.0184 |

Superscripts denote statistically significant differences, $p < 0.05$

CE= 1.64mL Control Extract (extract without rumen fluid); E= 1.64mL Extract (extract with rumen fluid); 1GP= 1g Purple-Fleshed Sweetpotato Powder