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Cover: The cover illustration was drawn by Jonathan Frye, using homemade *Acer saccharinum* charcoal.

The quotation is taken from Wendell Berry's essay, *The Loss of the University*, (p. 82 in *Home Economics*, North Point Press, 1987) in which he references the book *Samuel Johnson*, by W. Jackson Bate (Harcourt Brace Jovanovich, 1977, p. 51) as follows: "Dr. Johnson told Mrs. Thrale that his cousin, Cornelius Ford, `advised him to study the Principles of everything, that a general Acquaintance with Life might be the Consequence of his Enquiries - Learn said he the leading Precognita of all things ... grasp the Trunk hard only, and you will shake all the Branches.'"

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The Effects of Vitamin A on Gene Expression of BAX in MCF-7 Breast Cancer Cell Lines

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ABSTRACT

Breast cancer is one of the most diagnosed malignancies found among women. Current treatment options are invasive and come with extensive negative side effects. In addition, an increase in drug-resistant breast cancer cells is preventing a number of those treatment options from working. Recent scientific literature has begun the exploration of vitamins and vitamin derivatives as possible treatment or preventative options for breast cancer. Research on Vitamin A specifically, showed that Vitamin A has protective effects that prevent damage and disease to one's body, and antioxidant properties that can aid in disease prevention. This research aimed to explore the effects of Vitamin A, specifically in its retinol or preformed form, on the gene expression of BAX, in the growth of MCF-7 breast cancer cell lines. BAX, an apoptotic gene was of interest because of its potential ability to target a breast cancer cell's death pathway. To test this, cells were treated with 0.5, 2, and 3.5 μM of retinol for two different periods, 24 and 48 h. A cell viability count was done using the trypan blue stain test. An MTT reduction assay was then used to assess the number of living cells of the different treatment and control samples in comparison to one another. The results from the MTT suggest that there was a decrease in living cells as dosage and incubation went up, however, confirmation that this was because of an overexpression of the BAX gene specifically was inconclusive.

Keywords: *Breast Cancer, MCF-7, Vitamin A, Retinol, BCL-2, BAX*

INTRODUCTION

Breast cancer continues to be one of the most diagnosed malignancies found in women ("Breast Cancer", 2024). The American Cancer Society estimates that there will be 310,720 new cases of breast cancer diagnosed and 42,250 total breast cancer-related deaths in 2024 alone ("Breast Cancer", 2024). There is a 1 in 8 chance that a woman in the United States will develop breast cancer at some point during her life ("Breast Cancer", 2024). Incidence rates have continued to increase by 0.6% per year since the mid-2000s ("Breast Cancer", 2024). Women younger than the age of 50 are also now being affected at higher rates than ever before ("Cancer Facts & Figures", 2024). Health professionals attribute a portion of this increase to excessive body weight gained through dieting and lifestyle choices, consumption of alcohol, excessive smoking, and hormonal imbalances ("Cancer Facts & Figures", 2024). However, the exact cause of many breast cancer cases is unknown.

Current treatment options for breast cancer include surgery, radiation, chemotherapy, and endocrine therapy, which adds, blocks, or removes hormones (Alamro, et al. 2021). The risks and side effects associated with these types of treatment options include increased risks of uterine cancer, osteoporosis, stroke, pulmonary embolism, and vision problems to name only a few (Alamro, et al. 2021). In addition to this, there has been an increase in drug-resistant breast cancer cells which is preventing current available treatment options from working. Because of this and the extensive list of negative side effects associated with current treatment options, there has been a search for alternative options to prevent and treat breast cancer (Alamro, et al. 2021).

Vitamin A is an essential micronutrient to human health (Gilbert, 2013). It is a fat-soluble vitamin that plays a significant role in a number of biological functions. Some of these functions include the growth and development of cells, immune function, cellular communication, and reproduction in both males and females ("Vitamin A and Carotenoids", 2023). Research also indicates that Vitamin A influences cell proliferation, growth, and the differentiation of cancer cells (Marshall, 2000). Vitamin A comes from two major sources: preformed Vitamin A (retinoids) and provitamin A (carotenoids) (Kim, Ja-Hyun, Soo-Youn, 2021). Retinoids come from animal sources such as meats, dairy products, and eggs, while carotenoids come from plant sources and are pigments, usually red, orange, and yellow, that can be converted to Vitamin A in the intestine of the human body (Kim, Ja-Hyun, Soo-Youn, 2021).

Recent literature on Vitamin A, retinoids, and carotenoids has explored their relation to various types of cancer because of the suggested role that Vitamin A plays in regulating cell growth and differentiation ("Vitamin A and Carotenoids", 2023). Research shows that Vitamin A has protective effects and antioxidant properties. These characteristics are what aid in disease prevention, protect the body from

damage, and help get rid of free radicals (Kim, Ja-Hyun, Soo-Youn, 2021). Retinoids specifically have been noted to reduce the growth of malignant cells through targeted apoptosis (Kim, Ja-Hyun, Soo-Youn, 2021). Several epidemiological studies showed that higher concentration levels of carotenoids and retinoids in the body had an inverse relationship with the risk of acquiring invasive breast cancer in pre-and post-menopausal (Kim, Ja-Hyun, Soo-Youn, 2021).

This research focused on the mechanism of apoptosis. Apoptosis is the programmed death of a cell and serves as an important biological process, that protects cells against tumor development (Ola, Nawaz, Haseeb, 2011). Apoptosis is an important mechanism that is critical to normal cell population maintenance. In many cancers, the ability of a cell to commit apoptosis can become blocked or inhibited which can lead to invasive and hard-to-treat cancers.

This research looked specifically at the pro-apoptotic gene, BAX. BAX belongs to the B-cell lymphoma-2 (BCL-2) family of proteins located in the outer mitochondrial membrane. BCL-2 family proteins are responsible for regulating the mechanism of apoptosis within a cell (Yip and Reed, 2008). BAX specifically promotes apoptosis within the apoptotic pathway (Yip and Reed, 2008). BAX has been identified as a target for therapeutics because of its role in triggering apoptosis (Edlich, 2018). Targeting and regulating BAX gene expression could help to enhance healthy cell survival and promote apoptosis in malignant cells.

The MCF-7 breast cancer cell line was used as the cell line for experimentation. MCF-7 is the most commonly used cancer cell line when researching potential anticancer drugs and various treatment methods. There has been more research and data that has been produced by MCF-7 compared to any other breast cancer cell line that currently exists. (Comşa, Anca, Marius, 2015). MCF-7 is progesterone receptor-positive (PR+) and estrogen receptor-positive (ER+) which classifies it as a luminal subtype, meaning that the cancer starts at the inner lining of the mammary ducts (Comşa, Anca, Marius, 2015). It is also classified as a non-invasive line because the cells are localized to only the breast tissue (Comşa, Anca, Marius, 2015).

The negative side effects associated with current breast cancer treatment options and the increase in drug-resistant cancer cells have propelled research for alternative and safer treatment options (Alamro, et al. 2021). Because of Vitamin A's biological benefits and antioxidant properties, this research explores how it affects the gene expression of BAX, a pro-apoptotic gene, in the growth of MCF-7 breast cancer cell lines.

MATERIALS AND METHODS

Cell Culturing

The cells used for this experiment were donated by Athanasia Panopoulos' lab at Cedars-Sinai. The cells were cultured on tissue culture plastic (TCP) because of their anchorage dependency. The cells were grown on 6-well plates at an environment of 37 °C with 5% CO_2 levels. They were maintained in 2 mL of sterile media that consisted of Dulbecco's Modified, Eagle Medium (DMEM) that was supplemented with 10% fetal bovine serum (FBS). Every third day the medium was changed.

Once cells were at least 90% confluent across the plate, subculturing took place. For subculturing, the cells were washed with PBS to remove any traces of leftover serum. 1 mL of 0.5% trypsin-EDTA was then added to the wells to detach cells from the plate. The plate was incubated at 37 °C with 5% CO_2 levels for 10 minutes to further dislodge any attached cells. Cells were transferred to a sterile 15 mL conical and centrifuged at 1000 x g for 5 minutes to form a pellet. The supernatant was discarded, and fresh media (2 mL per new well) was added to the conical to break apart the pellet. Cells and the fresh media from the conical were transferred over to a new sterile 6-well plate for further culturing.

Vitamin A - Retinol Treatment

Treated cells were exposed to concentrations of 0.5, 2, and 3.5 μM retinol for two different incubation periods (24 or 48 h).

Cell Counting - Trypan Blue Stain Test

The Trypan Blue Stain Test is a dye exclusion test, that stains only dead cells whose cell membranes have been broken. Live cells, whose membranes are still intact, will remain unstained.

The protocol for subculturing above was followed up until plating, and instead, the pellet of cells in the 15 mL conical was resuspended in 1 mL PBS. A 10 μL sample of the cells and PBS mixture was taken and combined with 10 μL of sterile filtered 0.4% trypan blue in a new 1.5 mL microcentrifuge tube ("Trypan Blue Staining Assay").

A 10 μL drop of the 1:1 mixture of cells and trypan blue stain was added to a hemocytometer to manually count the number of living and dead cells. The percentage of viable cells was determined using the equation (number of viable cells / total number of cells) x 100. The average number of cells per square was calculated using the equation (viable cells) / (squares counted on hemocytometer). The dilution factor was calculated using the equation (final volume) / (volume

of cells). The number of cells present was calculated by taking the (average number of cells per square) (dilution factor) (10^4).

MTT Reduction Assay

The MTT Reduction Assay assessed cell viability of the 0.5 µM, 2 µM, and 3.5 µM retinol treatments at 24 h and 48 h incubation periods in comparison to one another and their respective control groups.

MCF-7 cells were plated on a 96-well plate so that there were approximately 20,000 cells per well. After three days from plating, the cells were 70% confluent and ready for treatment. The 48-hour treated cells were given their corresponding dosage of retinol (0.5 µM, 2 µM, and 3.5 µM). The next day, the 24-hour treated cells were then given their corresponding dosage of retinol (0.5 µM, 2 µM, and 3.5 µM).

After treatment, the cells were ready for analysis. All media was removed from the wells and the cells were washed with PBS. Then 50 µL of DMEM media containing 0.5 mg/mL of MTT reagent (Thiazolyl Blue Tetrazolium Bromide), a yellow color, was added to each well. The plate was incubated for 3 hours at 37 °C. Following incubation, 150 µL of Dimethyl sulfoxide (DMSO) was added to each well and mixed gently avoiding any bubbles.

The absorbance value was measured at 570 nm using a microplate reader. The cell viability percentage was calculated by taking (absorbance value of experimental sample / absorbance value of control sample) x 100. The cell toxicity percentage was calculated by taking ((absorbance value of control sample - absorbance value of experimental sample) / absorbance value of control sample) x 100.

BCA Protein Assay

A bicinchoninic acid (BCA) assay was used to measure the protein concentration of treated cells to ensure that all samples were the same concentration before performing the western blot. Knowing the protein concentrations allowed for accurate quantification of protein levels in the western blot. The kit used was the “Pierce BCA Protein Assay” by Thermo Fisher Scientific.

Once the total volume had been established, 50 parts of BCA Reagent A were mixed with 1 part of BCA Reagent B. Then 25 µL of each standard and unknown sample replicate was added into the microplate wells. Each sample was run as a triplicate. Each well then received 200 µL of working reagent (WR). The plate was mixed lightly and incubated for 30 minutes at 37 °C. The plate was measured at an absorbance value of 562 nm.

Western Blot Analysis

The western blot analysis was used to identify the presence of BAX in the treated cells. The cells were first treated with a radioimmunoprecipitation (RIPA) lysis buffer to disturb and break apart the cell membrane to expose all of the proteins. The RIPA lysis buffer was specifically chosen because of its recommended use for the extraction of cytoplasmic and nuclear protein when working with mammalian cells or soft tissue.

After lysis, for each treatment, 25 µg of protein, and equal parts of Laemmeli gel sample buffer, including 2% beta-mercapto-ethanol, were combined, and heated for 5 minutes at 95°C in 1.5 mL screw cap microcentrifuge tubes. The western blot apparatus (The Mini-PROTEAN Tetra Cell) was assembled for running a gel and 1x running buffer was added.

A 1.5 mm polyacrylamide gel was poured, and the wells were loaded in the following order: molecular weight ladder, 24 h control, 0.5 µM – 24 h, 2 µM – 24 h, 3.5 µM – 24 h, 48 h control, 0.5 µM – 48 h, 2 µM – 48 h, 3.5 µM – 48 h.

The electrode apparatus lid was placed on and the box was connected to the power supply. The gel was run for 1 hour at 200 V and 42 mA so that the bands were just near the bottom of the gel.

After the gel finished running, it was then ready for transfer. A “transfer sandwich” containing the gel, nitrocellulose membrane, blotting paper, and sponge was enclosed in a cassette and soaked in a transfer buffer.

The “transfer sandwich” was then placed into the transfer apparatus chamber (Mini Trans-Blot Electrophoretic Transfer Cell) with an ice pack and stir bar. The transfer buffer was added to the apparatus before being connected to the power supply. The transfer ran overnight at 30 V 90 mA.

Following the overnight transfer, the nitrocellulose membrane was removed and placed in a dish with a blocking buffer, containing 4% milk. This was incubated overnight at 4°C.

A 1:20 dilution of primary antibody (BAX Monoclonal Antibody 6A7 from Thermo Fischer Scientific) in blocking buffer was then incubated with the membrane overnight at 4°C.

The membrane was washed in washing buffer before incubating in a 1:10,000 dilution of the secondary antibody (Goat anti-Mouse IgG H+L Poly-HRP) and blocking buffer for 1 h at room temperature.

The membrane was washed in washing buffer and treated with Chloronaphthol, a chromogenic substrate that promotes band visibility in western blot analysis to allow for band detection.

RESULTS

Vitamin A - Retinol Treatment

MCF-7 cells were imaged at 10x magnification using a Zeiss AX-10 inverted lens. In both the 24 h and 48 h wells, the cells were nearly confluent across the entire plate as shown in Figure 1.

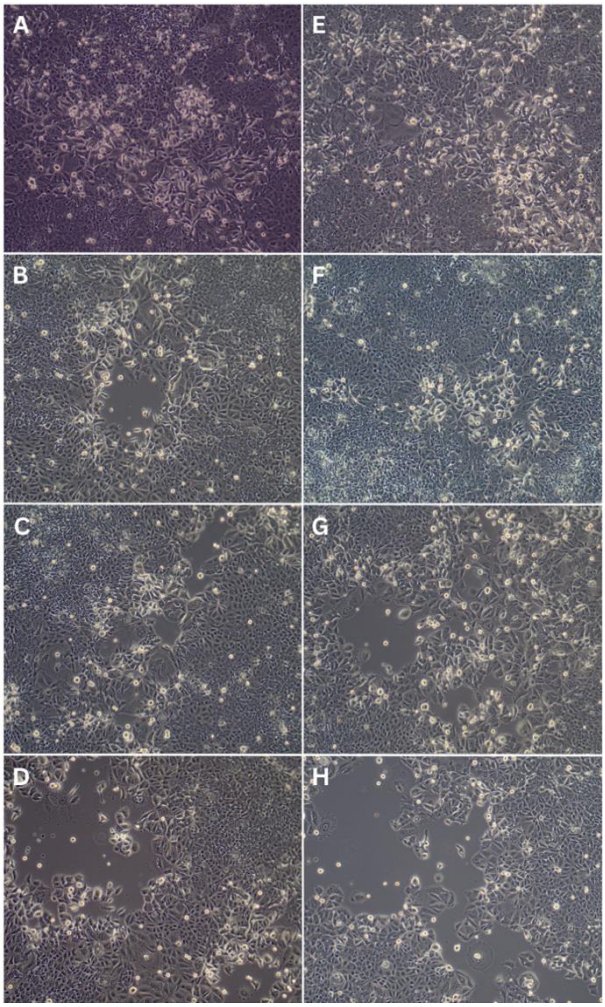


Figure 1. A) Control 24 h cells. B) 0.5 µM 24 h cells. C) 2 µM 24 h cells. D) 3.5 µM 24 h cells E) Control 48 h. F) 0.5 µM 48 h cells. G) 2 µM 48 h cells. H) 3.5 µM 48 h cells.

In the treated 3.5 µM 24 h and 48 h wells (Images D and H of Figure 1), there were noticeable areas of diminished cell growth that formed toward the center of the plate. These cells were plated and cultured in the same conditions prior to treatment. This suggests that 3.5 µM in both 24 h and 48 h treatments caused some cells to die and become unattached to the plastic.

Trypan Blue Stain Test

The Trypan Blue Stain Test revealed the number of viable cells and the percentage viability provided in Table 1. Because this test does not ensure that the taken sample has equal cell counts before staining, an MTT reduction assay is needed to assess cell viability after treatment in wells with the same cell counts.

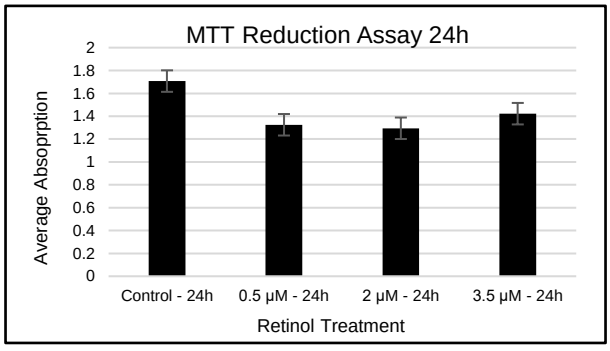
Table 1. The eight conditions (six retinol treatments and two control groups) and the number of viable cells and their percentage viability.

Trypan Blue Stain Test (24 h Samples)		
Conditions	**Number of Viable Cells**	**% Viability**
Control 24 h	1,360,000	94%
0.5 µM 24 h	1,210,000	92%
2 µM 24 h	1,040,000	95%
3.5 µM 24 h	1,280,000	93%

Trypan Blue Stain Test (48 h Samples)		
Conditions	**Number of Viable Cells**	**% Viability**
Control 48 h	1,000,020	95%
0.5 µM 48 h	1,229,976	93%
2 µM 48 h	1,653,300	92%
3.5 µM 48 h	1,110,000	93%

MTT Reduction Assay Dosage Comparison

For the MTT Reduction Assay, triplicate samples were run for each condition on a 96-well plate. These cells were plated at about 20,000 per well as stated in the Methods section, which is what allowed their absorbance values to be comparable with one another as shown in Figure 2.



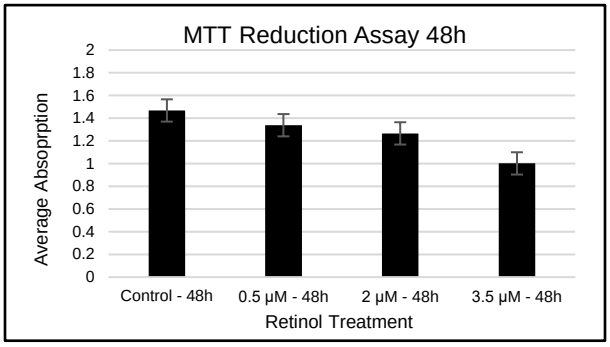


Figure 2. The two graphs display the absorption values that were calculated from the MTT Reduction Assay after dosages of 0.5, 2, and 3.5 µM concentrations of retinol for 24 h and 48 h incubation periods. The standard error is represented by the extending line from the bar.

In the 24 h samples, all treatment groups had less metabolically active cells when compared to the control group's average (1.707). The 3.5 µM treatment group under the 24 h conditions, was the only absorption value to not decrease less than the previous dosage of 2 µM. The triplicate of the 3.5 µM for 24 h, had values that were not closely related which did affect the overall average used.

In the 48 h samples, as the dosage of retinol increased, the number of living cells also decreased. Notably, the 3.5 µM of retinol dosage for the 48 h incubation condition group has the lowest average absorption value (1.002), when compared to the control 48 h average (1.468). This suggests that out of all the treatment groups, a dosage of 3.5 µM of retinol for an incubation period of 48 h is the most effective at decreasing the number of metabolically active cells in a sample.

Furthermore, Table 2 shows the toxicity and cell viability percentages calculated for each of the treatment samples based on the respective control.

Table 2. MTT Reduction Assay cell toxicity and viability percentages in comparison to the control groups.

Treatment	Toxicity	Viability
0.5 µM 24 h	22.37%	77.63%
2 µM 24 h	24.14%	75.86%
3.5 µM 24 h	16.70%	83.30%
0.5 µM 48 h	8.86%	91.14%
2 µM 48 h	13.76%	86.24%
3.5 µM 48 h	31.74%	68.26%

The data from the MTT assay suggests that as the dosage of retinol and incubation time increases, the number of cells growing decreases. Since the cells were plated at the same density and grown under the same conditions, more cells underwent apoptosis and faced higher cell toxicity, as the treatment of retinol and incubation period increased. A western blot was needed to confirm that this decrease was because of an increase or overexpression of the BAX gene.

BCA Protein Assay

A BCA protein assay was run before the western blot to ensure that equal amounts of protein were added to the gel wells.

A standard curve was generated based on a set of standards, and protein concentrations were calculated for each group.

Table 3. Column 1 lists the respective condition of the sample. Column 2 displays the absorption value that is calculated from the microplate reader. Column 3 calculates the corresponding protein concentrations calculated from the line equation produced from the standard curve (24 h, x=(y-0.0502)/0.0009) (48 h, x=(y-0.1282)/0.0009).

Condition	Absorption Value Based on Standard Curve	Protein Concentration
Control 24 h	0.362	346 µg
0.5µM – 24 h	0.330	311 µg
2 µM – 24 h	0.289	265 µg
3.5µM – 24 h	0.410	400 µg

Condition	Absorption Value Based on Standard Curve	Protein Concentration
Control 48 h	0.843	794 µg
0.5µM – 48 h	1.266	1264 µg
2 µM – 48 h	1.471	1492 µg
3.5µM – 48 h	1.45	1469 µg

Western Blot Results

After multiple attempts to get band imaging, the membrane was not able to provide bands that confirm, nor deny, an overexpression of BAX in the higher dosage and incubated samples when compared to the control groups.

DISCUSSION

Other scientific literature and research projects on vitamin and vitamin derivatives show a similar trend, as shown in the MTT results from this experiment. One study looked at the effects of vitamin E compounds on breast cancer (Kline, Weipingm, Sanders, 2004). There were five of the vitamin E compounds that were shown to have selectively induced apoptosis in cancer cells (Kline, Weipingm,

Sanders, 2004). Another study looked at the effects of vitamin C in high dosages in combination with other current treatment options like tamoxifen and trastuzumab (Lee, et al. 2019). Through MTT and other cell viability assays, this research also determined that there was a decrease in cancer cell proliferation using high dosages of vitamin C even in combination with other standard treatment options (Lee, et al. 2019). Additionally, several studies have explored the effects of vitamin D on different breast cancer cell lines. In a study done on mice, certain biological properties of vitamin D slowed the cancer cell growth, decreased further blood vessel formation at the cancer sites, and eventually were able to induce apoptosis in response to vitamin D dosage levels (Williams, et al. 2016).

Regarding vitamin A specifically, there is research and data that supports how vitamin A deficiencies can lead to an increased risk of developing breast cancer, but there is no other data on the actual effects on cancer cell growth when used as a treatment option ("Vitamin A and Carotenoids", 2023).

The recommended dietary allowance for Vitamin A in women 50 years and older is 700 mcg of retinol activity equivalents (RAE) ("Vitamin A and Carotenoids", 2023). A normal plasma concentration of retinol has a range from about 0.5-3 μM / L. The 3.5 μM is right outside of the normal plasma concentrations ("Vitamin A and Carotenoids", 2023). The upper limit of retinol, however, is near 10 μM concentration ("Vitamin A and Carotenoids", 2023). This leaves a large area outside of normal and before the upper limit that is gray in terms of the effects of a 3.5 μM retinol concentration. The concentrations used in this research were selected based on the above information as well as other similar vitamin studies ranges.

Because the western blot was not successful in detecting bands, it is unknown whether the decrease in metabolically active cells observed in this research was because of overexpression of BAX specifically. Regardless, the ability to regulate the BAX complex in cancer-specific cells is a focus area of many targeted therapy research projects.

In this research, one possible reason for the lack of bands on the membrane for the western blot could be related to the amount of protein being added to the wells of the gel. In the first western blot, only 5 μg of each sample was added to the wells. The second time running the analysis there were 25 μg of each sample added, yet still no bands were observed.

A more likely issue could be in the steps during transfer. One recommended solution to ensure that proteins have successfully transferred over to the membrane from the gel is to run a Ponceau staining. Ponceau staining is an extremely sensitive stain that has the ability to detect low amounts of proteins present on the membrane and in the gel. This stain also does not alter the integrity of the proteins themselves, and the membrane can be fully de-stained.

One other possible error that prevented band imaging could be over-washing or over-blocking the membrane. The overnight protocol was followed for each of the probing steps (excluding the secondary antibody), however since a smaller membrane was used in order to be compatible with the Mini-PROTEAN Tetra Cell apparatus, potentially the hour protocol could have been the better choice to prevent over-washing or over-blocking.

The final area of potential error could have been in the age of reagents used. For example, chemicals like Tween-20 (used in the wash buffer), Triton-X (used in the RIPA buffer), and Beta-Mercapto-Ethanol (used in the sample buffer), all have a recommended shelf life of under four years if stored properly. These chemicals were significantly older than four years and were potentially not as effective in their respective buffers.

It is unlikely that this issue has anything to do with my primary or secondary antibody. The recommended amounts were followed for both antibody concentrations. Additionally, both are compatible with the cell line being used.

Although the overexpression of the BAX complex was not able to be identified through a Western Blot analysis, nonetheless, the MTT provided data that suggested that there were fewer living cells in the samples treated with higher concentrations of retinol for longer incubation periods. This suggests future areas of research in determining what the specific cause is for the decrease, as well as expanding the range of dosage and exposure periods to find the most optimal level of cancer cell death. Furthermore, it is important to consider and look at what effects these concentrations and exposure to retinol have on normal luminal breast tissue since, in most treatment options, the surrounding healthy tissue can still be affected.

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Observing and Comparing the Cortisol Concentrations in Weaning Calves Before and After the Weaning Process

By Maggie Brown

ABSTRACT
Many things can be the cause of stress in calves near the weaning age, but the process of weaning itself may be the most stressful. The goal of this study was to observe the concentrations of cortisol in the saliva of weaning calves during different stages of the process. It was carried out over a seven-week period through August and September of 2024. The study was carried out on 10 Black Angus calves on Aero B Ranch in northwestern Kansas. A total of 8 samples from each of the calves was collected. After all collections were complete, the samples were analyzed using an ELISA Kit to find the optical densities of each sample compared to standards sent with the kit. The optical densities were converted to cortisol concentrations and those concentrations were compared using a repeated measures ANOVA test. It was revealed that there is a significant difference in cortisol concentrations at different points, specifically two days post weaning, during the weaning process.

Keywords: *weaning, calves, salivary cortisol, ELISA, ANOVA.*

INTRODUCTION

Every year the time comes for ranchers to separate the cow-calf pairs in the herd. This process is referred to as weaning. Weaning is unavoidable and is typically very stressful, for the cow and the calf (Kohari et.al., 2014). For the calf, the process of weaning can be the beginning of a long chain of difficult, potentially lethal, events. During the course of this process, calves are losing their mothers, getting vaccinations, receiving a new feed source, and most likely being moved to a new location (Comerford, 2022).

Calves can be weaned at varying ages, depending on many factors including the cow's body condition, sale times, and other normal ranch activities. As long as the calf's rumen has become functional – meaning that its digestive system can process whole feeds – it can be weaned. Generally, calves are separated from their mothers anywhere between three and eight months old, however, bottle-fed calves can be weaned at as little as one month old. Weaning during this time allows the mother a small downtime to preserve energy before her re-breeding (Cothren, 2021).

Stress is typically known as the "fight or flight response." More specifically, stress is a feeling or symptom that is caused by a non-normal environment or situation. Like humans, cattle undergo varying amounts of stress, and depending on the degree, it can negatively affect them for significant periods of time. Some of these negative effects include a weakened immune system, weight loss, digestion issues, lack of appetite, and inflammatory reactions. The main indications of stress are respiratory issues, abnormal behavior, trembling, and lack of coordination. The level of stress can be measured by observing the amount of the stress hormone cortisol in a calf's saliva or blood (Hernandez et.al., 2014). The stress response is initiated in the brain, at the amygdala. A signal is sent from the amygdala to the hypothalamus, the command center of the brain. When the amygdala continues to sense a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH), which triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. When ACTH reaches the adrenal glands, they are prompted to release cortisol, and the body stays in the "fight or flight" response until the threat passes (Harvard Medical School, 2020). Measuring the cortisol levels in saliva has been used as a less invasive alternative to sampling blood.

It is important to measure the stress in calves for a couple of reasons; one reason being that ranchers always hope to have healthy and thriving herds, and it is impossible to have that without a little background knowledge of what your calves face during the weaning season. The second reason is that the effects of stress can lead to death, in some cases, leaving a wake of disruption behind it. The rancher loses livestock, which starts a chain of loss: the loss of a sale, the loss of money from that sale, and the potential to lose so much more. This research can help ranchers determine how long they will need to keep a closer eye on their weaned calves and be more aware of the signs of stress (Eilerts, 2020).

As a rancher myself, my curiosity about the stress on the calves during and after the weaning process brought me to this question: How long do stress levels in calves stay elevated after weaning? Throughout the duration of this project, I intend to collect saliva samples from multiple calves before the weaning process, during the weaning process, and every week after the weaning process until the cortisol levels return to or get close to the pre-weaning level. It is anticipated that the cortisol levels will return to the pre-weaning level in less than one month, specifically, three weeks.

MATERIALS AND METHODS

During the course of the research, salivary cortisol

from ten Black Angus calves living at Aero B Ranch in Wallace, Kansas will be sampled, a total of eight times, and examined. The calves are all between five and six months old. There are two heifers and eight steers within the group of calves, two of the calves are from different sets of twins.

Animal Housing and Management

The sampling was completed on Aero B Ranch in Wallace, Kansas. The ranch has roughly 70 Black Angus cows that have been bred by Black Angus bulls. The untested cow-calf pairs are currently kept in a large pasture just south of the ranch house, this is where all of the cattle were kept until shortly before the weaning procedure. After weaning, the cows were moved to a pasture north of the house that is adjacent to the corral where the calves were residing. The calves were still able to see and hear their mothers, but they were not able to physically reach them. The cows have been eating mostly pasture grass, but they have always had access to a hay bale as well. The calves have been fed corn silage once a day in the concrete bunks that are next to their corral, they also always have access to a hay bale. Both the cows and calves have always had access to the water tank.

Sampling Procedures

The sampling was conducted on 10 calves over seven weeks during August and September 2023. Starting on 8/17/23 and ending 9/27/23. The saliva samples were collected two weeks before weaning, one week before weaning, on the day of weaning, two days post-weaning, and every weekend after that for the next three weeks. All weaning was completed on the same day, so the calves were of various ages. On the day of the weaning, the calves were run through the chute with their mothers leading them and they were sampled to receive the last of the baseline samples. A study performed on dairy cattle in Sweden in 2014 has shown that the peak of cortisol levels in saliva has a roughly ten-minute time lag from the peak cortisol levels in the blood, so that is important to take into consideration during future experimentation. Their experiment involved the simultaneous collection of blood and saliva so that the samples could be directly compared to one another (Hernandez, et.al., 2014).

That same day as the last baseline, the calves were run back through the chute after they had been separated from their mothers, and they were resampled to collect the initial spike in stress. The next sample was collected two days after weaning by family members and was kept in the freezer until the next weekend. Every weekend, for the next three weekends, samples were collected. All sampling was completed by Maggie Brown and Brad Brown, with the help of Cordell Brown, Jacob Banman, Xavier Hernandez, and Nate Brown moving all of the cattle at the appropriate times.

All sampling was completed in the mornings when the afternoon temperature had not yet arrived. This was to avoid heat stress on the calves, which could sway the results of the testing. Each sample was taken using a 15mL plastic test tube and the catch method. Water was pushed into each calf's mouth using a 12mL syringe so that the saliva could gently fall out of their mouths. Immediately after collection, the tubes were stored on ice until all collections were complete. Then the collection tubes were centrifuged, and the supernatant was decanted into other tubes that were frozen until the cortisol analysis.

Analysis Procedures

The cortisol from the saliva was analyzed using a Cortisol Saliva ELISA Assay Kit from Eagle Biosciences (Eagle Biosciences, n.d.). ELISA Assay Kit and all instructions of the kit will be followed. The optical densities of each sample were measured during the ELISA analysis using a microplate reader. The optical densities were then converted to cortisol concentrations in ng/mL using the given concentrations of the standards provided in the kit. The calculated concentrations were then analyzed using a repeated measures ANOVA in the Jamovi Statistical Analysis Package (Jamovi Version 2.4.6., n.d.)

RESULTS

After the completion of the Cortisol Saliva ELISA Assay Kit, the standard curve of the optical densities was constructed. (Table 1) The line's equation was used to convert the measured optical densities to cortisol concentrations in ng/mL.

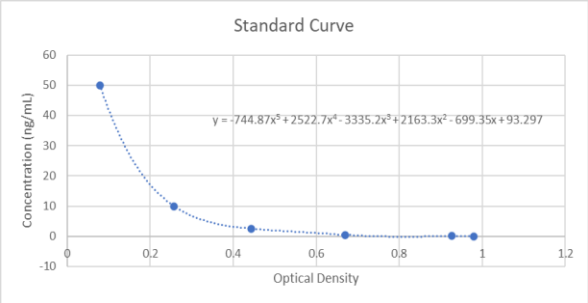

Figure 1: The Standard Curve Calculated from the Standards provided in the ELISA Kit. (A Polynomial to the 5th order) Equation: y = -744.87x^5 + 2522.7x^4 - 3335.2x^3 + 2163.3x^2 - 699.35x + 93.297

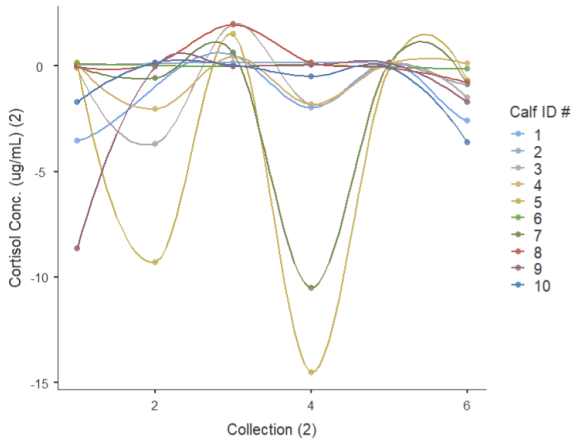

Figure 2: Cortisol Concentrations from Each Sample at Each Collection Date (The pre-weaning collections have been averaged) – This data also excludes some data points from the Day of Weaning so that a better look at the graph can be observed.

There was a significant difference in the cortisol concentrations of the calves across the testing periods ($F_{5,45}$ = 2.49, P = 0.045). A post-hoc analysis of the testing periods shows that the significant difference in the cortisol concentrations was specifically at 2 Days Post-Weaning. Tukey's Honestly Significant Difference (HSD) Test (Table 1) indicates that the only differences across the sampling period were between Level 1 and Level 3 and then Level 5 and Level 6. This shows that there was a significant spike in the cortisol concentration of all the calves on the two days post-weaning collection, but cortisol levels declined back to pre-weaning levels by Level 4 (Figure 2).

Post Hoc Comparisons - RM Factor 1

Comparison							
RM Factor 1		RM Factor 1	Mean Difference	SE	df	t	P_{tukey}
Level 1	-	Level 2	172.578	109.385	9.00	1.578	0.630
	-	Level 3	−2.089	0.824	9.00	−2.534	0.209
	-	Level 4	1.708	2.060	9.00	0.829	0.954
	-	Level 5	−1.422	0.904	9.00	−1.573	0.633
	-	Level 6	−0.103	0.797	9.00	−0.129	1.000
Level 2	-	Level 3	−174.667	109.415	9.00	−1.596	0.620
	-	Level 4	−170.870	109.835	9.00	−1.556	0.642
	-	Level 5	−174.000	109.491	9.00	−1.589	0.624
	-	Level 6	−172.680	109.434	9.00	−1.578	0.630
Level 3	-	Level 4	3.797	1.718	9.00	2.209	0.319
	-	Level 5	0.667	0.261	9.00	2.557	0.203
	-	Level 6	1.986	0.397	9.00	4.999	0.007
Level 4	-	Level 5	−3.130	1.621	9.00	−1.931	0.443
	-	Level 6	−1.811	1.732	9.00	−1.045	0.891
Level 5	-	Level 6	1.319	0.362	9.00	3.641	0.044

[4]

Table 1: Tukey's Honestly Significant Difference Test displays that the largest significant difference is within Level 3. (Level 1: Pre-Weaning Averaged Concentrations, Level 2: Day of Weaning Concentrations, Level 3: Two Days Post-Weaning Concentrations, Level 4: One Week Post-Weaning Concentrations, Level 5: Two Weeks Post-Weaning Concentrations, Level 6: Three Weeks Post-Weaning Concentrations.)

DISCUSSION

The goal of this study was to observe the concentration of cortisol in the saliva of calves during different stages of the weaning process and test the hypothesis that weaning does increase the stress level of the calves and it remains elevated for a couple of weeks. On Aero B Ranch, in Wallace, Kansas, ten calves were selected and had saliva collected on eight occasions. In response to the stress of being separated from their mothers, I expect that each calf will show an initial increase in its salivary cortisol concentration, and then with time, its concentration level will decrease. After completing the Salivary Cortisol ELISA Kit instructions and reading the samples on a microplate, I found that there was a spike in the cortisol concentration following the weaning itself. However, the concentration did not remain elevated as long as initially estimated.

Cortisol is commonly known as the "stress hormone." However, it has many effects and functions throughout the body that are more than just regulating the body's stress response. Almost all of the tissues in the body have glucocorticoid receptors, so cortisol can affect almost every organ system in the body; this includes: the nervous system, the immune system, the cardiovascular system, the respiratory system, the reproductive system, the musculoskeletal system, and the integumentary system. The effects of cortisol are also very broad; it can regulate the stress response, it can regulate metabolism, it can suppress inflammation, it can regulate blood pressure, and it can help control the sleep-wake cycle (Cleaveland Clinic, 2021). In cattle specifically, cortisol has been affiliated with lower reproduction rates, suppressed milk production, and suppression of the immune system that can cause greater susceptibility to diseases. Exposure to stressors in the short-term can lead to a sharp increase in the secretion of cortisol. Chronic stress is caused by the long-term presence of stressors, and this can lead to cells having a reduced sensitivity and response to cortisol (Villamediana, 2022).

As expected, no two calves displayed the same concentrations at each collection interval. The optical density readings show that the calves had not had an increase in cortisol concentration on the day of the separation. However, there was a significant spike in the cortisol concentration of all the calves on the two days post-weaning collection. The results from the one-week post-weaning collection displayed that the calves were recovering from their stress.There was a second, smaller, spike in almost all of the calves on

the collection that was taken two weeks post-weaning. This could have been caused by the weather conditions on the day of the sampling. On the final collection, roughly three weeks after the separation, the cortisol concentrations have reapproached the average of the pre-weaning baseline samples. This follows the initial hypothesis that the concentrations would return to baseline by the third week. (Figure 2) A strange phenomenon of some very negative cortisol concentrations was observed on the Day of Weaning (Figure 2). This could be due to a procedural incident while working with the ELISA Assay Kit or a malfunction with the microplate reader.

Through this research, it was found that the stress of the weaning process does in fact cause a spike in the salivary cortisol concentrations of the calves being weaned. It was also found that the length of time that the cortisol concentrations remain elevated was much shorter than expected to be, showing that cattle recover from this type of stressor very effectively or that the process of weaning is not as stressful as initially hypothesized.

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The Comparison of Vitamin C Content in Orange Juices Made by Various Methods

Shekinah Ilunga Manyonga

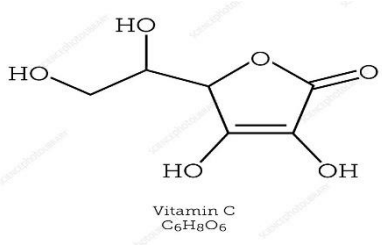
ABSTRACT

The goal of this research was to compare the Vitamin C concentration in 100% orange juice from various brands in comparison to fresh oranges. Vitamin C is a nutrient that the body needs to form blood vessels, cartilage, muscle, and collagen in bones. A redox titration was performed to accurately measure the vitamin C concentration. In our comparative analysis of vitamin C concentrations in orange juice, Minute Maid exhibited a remarkable alignment with both expected values and the vitamin C content found in whole oranges, indicating a potential fortification strategy to maintain consistency. We concluded that during juice extraction many important nutrients are left behind, opting for whole oranges instead of consuming 100% fruit juice is preferable because the pulp and skin of many fruits are rich in essential vitamins and nutrient.

Keywords: *vitamin c, orange juice, ascorbic acid, titration, iodine*

INTRODUCTION

Vitamin C, scientifically known as ascorbic acid, is a water-soluble vitamin. It is not stored in the body therefore it must be taken through food or supplements.



Vitamin C
$C_6H_8O_6$

The chemical structure of ascorbic acid determines its physical and chemical properties. It is a weak, unstable organic acid, which can be easily oxidized or destroyed, in light, aerobic condition (oxygen), high temperature, alkali, humidity, copper and heavy metals (Yussif 2018).

Vitamin C is an antioxidant with hydroxyl groups that donate electrons, effectively neutralizing harmful free radicals in the body. By preventing oxidative damage to cells, DNA, proteins, and lipids, this robust antioxidant action supports overall cellular health and may confer protective effects against chronic diseases (Nordqvist 2021).

Vitamin C helps make several hormones and chemical messenger used in the brain and nerves (T.H Chan 2020). However, too much of vitamin C in the body may lead to the person excreting the compounds oxalate and uric acid in their urine (Cupsis, Adamsco et al 2023) and too little vitamin C may lead to vitamin deficiency known as scurvy (Johnson 2023).

Vitamin C is widely distributed in fresh fruits and vegetables. Fruit juices come from the flesh of the fruits or from the whole fruit itself. The method of making fruit juice varies depending on the fruit, but many manufacturers make juices by crushing or pressing the fruit to squeeze out the juice inside, then pasteurizing or adding preservatives before packaging the final product (Rowden 2021). Heat treatment remains the most widely used method used to extend the shelf life of different juices; this can cause a decrease in the nutritional components such as vitamins, carotenoids, and bioactive compounds (Soural, Ivo et al 2022). However, during the process of manufacturing, manufactures can add ingredients like sugar, ascorbic acid, colorings etc., so when purchasing 100% fruit juice it is important that you educate yourself with facts to aid in selecting a healthy product made from 100% fruit juices (Rellinger 2013).

The best way to enjoy the amazing taste and health benefits of 100% juice is by taking the recommended daily amounts. According to Harvard school of public health, the Recommended Dietary Allowance for adults 19 years and older is 90 mg daily for men and 75 mg for women. For pregnancy and lactation, the amount increases to 85 mg and 120 mg daily. However, the number of fruits to take per day depends on the age, sex, height, weight, and level of physical activity. For women, the amount can also depend on whether you are pregnant or breastfeeding (My plate.gov). Many Americans do not meet the daily recommendations for fruit consumptions (2015 dietary guidelines committee 2015), but those children and adults who consume the recommended amount of 100% fruit juices are more likely to meet daily fruit goals (institutes of food technologist 2017).

Among essential nutrients, vitamin C stands out as a vital component known for its numerous health benefits. One common source of this vitamin is oranges, which can be consumed in various forms; this includes 100% orange juice and whole oranges. Both forms of consumption are rich in vitamin C.

People wonder if there is a real difference in how much vitamin C; we get from these two options. This

research project will be investigating if 100% orange juice and oranges have the same amount of vitamin C or if one is better than the other is. This knowledge is important because knowing which product gives more vitamin C can help us make healthier choices in our diets.

MATERIALS AND METHODS

For this experiment, we used 100% orange juices from four different brands and oranges obtained from Walmart, McPherson KS. Every week for one month, we bought one bottle from each brand. The brands were Minute Maid, Simply Orange, Florida's natural no pulp and Florida's natural with pulp.

Sample preparation

Oranges were peeled and 100g of the oranges were blended with 50ml distilled water and the juice was strained using cheesecloth. Afterwards distilled water was added to make a final solution of 100ml in a volumetric flask. The standard solution was prepared by crushing a 500mg of vitamin C from the brand spring valley and adding 10ml of 1M sulfuric acid and diluted it to 500ml using deionized water.

Starch and iodine solution preparation

Starch solution was prepared using 0.50g soluble starch and 50ml boiling water. We mixed it well, dissolved the starch, and allowed it to cool before using it for the titration. The iodine solution was prepared by dissolving 5g of potassium iodide and 0.268g of potassium iodate with 200ml of distilled water and 30ml of 3M sulfuric acid was added to it. We diluted it to 500ml. We mixed the solution and transferred it into a plastic bottle.

Titrations

A redox titration was conducted using iodine solution as the titrant. For the standard solution, we used a 25ml sample and added ten drops of the starch solution. The endpoint of the titration was determined by the first constant blue-black color. For the oranges and the orange juice, for each titration, we pipetted 25ml of sample in an Erlenmeyer flask and added ten drops of starch solution. The sample was titrated with the iodine solution and the end point was determined by the first constant brownish color. Using the average volume of the iodine solution, we calculated the number of moles of ascorbic acid in the reaction and derived its concentration.

Calculation method

The concentration of vitamin C was determined through the iodine redox titration. By using the chemical equation $I_2 + I^- \leftrightarrow I_3^-$ in this titration vitamin C reacts with I_3^- in the presence of water to yield to $C_6H_6O_6 + 3I^- + 2H^+$ The iodine redox titration method relies on the reaction between iodine and iodide, with vitamin C as the reducing agent. As vitamin C is oxidized during the titration, iodine is reduced, leading to the formation of triiodide. We monitored the amount of iodine consumed to calculate the vitamin C concentration.

RESULTS

The comprehensive analysis of vitamin C concentrations in 100% orange juice is explained through the visual representation in Figure 1 and the statistical insights provided in Table 1. Figure 1 displays the average vitamin C concentration in mg/100ml alongside the concentrations indicated on the labels of each brand, facilitating a direct comparison between measured and labeled values. Table 1 presents the results of one-way ANOVAs conducted on multiple aliquots per bottle and multiple bottles per brand. The statistical analyses in Table 1 explain the variability in vitamin C measurements within each brand, providing valuable insights into the consistency of concentrations across different samples.

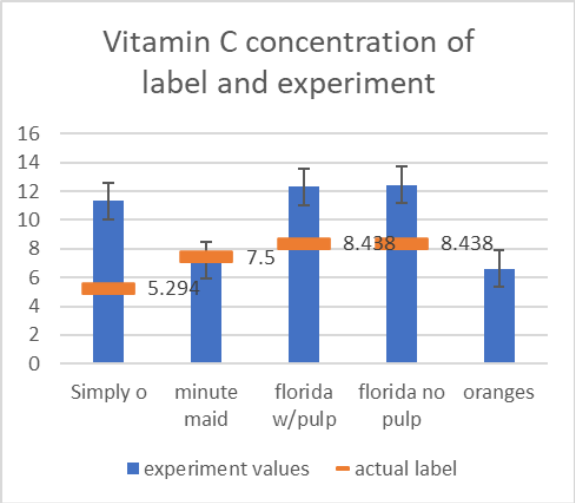


Figure 1: average vitamin C concentration in mg per 100ml of juice and the concentrations indicated on the label of each brand.

Table 1: Results of one-way ANOVAs of vitamin C measured in multiple aliquots per bottle and multiple bottles per brand of 100% orange juice.

The orange juice brand	df	SS	MS	F	p-value
Simply orange	2	131.43	65.72	168.44	0.0114

Minute maid	2	122.08	61.04	170.55	0.675
Florida's natural with pulp	3	416.07	138.69	209.78	0.0132
Florida's natural no pulp	3	211.77	70.59	95.76	0.00470

Considering the obtained results and the associated p-values, it's evident that the mean values for the brands Simply Orange, Florida's Natural with Pulp, and Florida's Natural with No Pulp were higher than the expected values. Notably, statistical analysis revealed a significant difference for all three brands: Simply Orange (p=0.011), Florida's Natural with Pulp (p=0.013), and Florida's Natural with No Pulp (p=0.005). However, when examining Minute Maid, the observed value aligns closely with the expected value, and no statistically significant difference was found between the means (p=0.675).

DISCUSSION

For this experiment we compared the vitamin C concentration of orange juice made by various methods. We were able to calculate the vitamin C concentration by performing a redox titration using iodine and starch solution. Iodine is insoluble, but was improved by complexing the iodine with iodide to form triiodide:

$I_2 + I^- \leftrightarrow I_3^-$

Triiodide oxidizes vitamin C to dehydroascorbic.

$C_6H_8O_6 + I_3^- + H_2O \rightarrow C_6H_6O_6 + 3I^- + 2H^+$

As long as the solution contains vitamin C, triiodide continues to be converted into iodide ion. Once all the vitamin C is oxidized, the iodine starts reacting with the starch present in solution forming a blue-black starch-iodine complex. The color changes indicate the endpoint of the titration. For this experiment the endpoint was a brownish color.

According to the results obtained, the vitamin C concentration for the brands simply orange, Florida's natural with pulp and Florida's natural with no pulp had a higher vitamin C concentration than the expected vitamin C concentration. However, we obtained the same amount of vitamin C concentration as the expected value for the brand Minute Maid. The same amount of vitamin C was obtained for oranges too.

The observed higher vitamin C concentrations obtained through the redox iodine titration in juices compared to the nutritional labels could be attributed to the oxidation of ascorbic acid during the titration process. The redox reaction involves the conversion of ascorbic acid to dehydroascorbic acid in the presence of iodine generating iodide ions. This oxidation of ascorbic acid results in an increase in the measured vitamin C concentration.

Furthermore, the sensitivity of the redox iodine titration method to ascorbic acid oxidation underscores the importance of considering the analytical approach in interpreting results. Other titrimetric methods or analytical techniques may offer different outcomes, depending on their selectivity and reaction conditions. The potential influence of oxidation on the measured vitamin C content should be acknowledged when comparing experimental results to nutritional labels. Additionally, variations in sample preparation, storage conditions, and the presence of interfering substances could contribute to disparities between measured and labeled vitamin C concentrations.

During manufacturing, some ingredients are added in amounts exceeding the label claims to compensate for expected losses during shelf life (Andrews et al 2016). This is done to account for potential losses that may occur over time due to factors such as air, temperature changes during storage and transportation. The measured concentrations in the experiment may reflect the initial excess quantities added during production, surpassing the labeled values.

In cases of minute maid, where the observed vitamin C concentration aligns with the nutritional label, it is possible that this brand employs a production process that maintains the natural balance of nutrients found in oranges. Some juice products are fortified with vitamins (Turner 2020). Minute Maid might use vitamin C fortification to achieve a consistent concentration, resembling that of fresh oranges.

In conclusion, opting for whole oranges instead of consuming 100% fruit juice is preferable because the pulp and skin of many fruits are rich in essential vitamins and nutrients. When only the juice is extracted, a massive portion of these valuable nutrients are left behind (Baird, 2013). It is worth noting that manufacturers often add a considerably higher amount of vitamin C than the labeled value suggests, aiming to compensate for the nutrient losses resulting from degradation (Methrom, 2004).

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Efficacy of Ampicillin and Kanamycin against Coliforms and Escherichia coli in Local Wastewater

Madison Turley

ABSTRACT

The development of bacterial resistance to antibiotics remains one of the greatest threats to public health, and communities are seeking to find ways to combat resistance. Unfortunately, antibiotic disposal from pharmaceutical settings, agricultural waste, or ineffective wastewater treatment drives gene development in bacteria to resist antibiotics. *Escherichia coli*, or *E. coli*, naturally occur in these environments and may confer resistance to or inactivate antibiotics by producing extended-spectrum beta-lactamases (ESBLs). To study resistance in locally treated wastewater in McPherson, Kansas, samples were collected before, during, and after the treatment process. Their physical properties and resistance to ampicillin and kanamycin were recorded, including total coliforms and *E. coli*, respectively. The samples revealed moderate resistance to ampicillin, characterized by little to no reduction of colony abundance, and a slight resistance to kanamycin, with abundance nearly eradicated. Results also indicated a significant reduction in colony count due to the ultraviolet disinfection of wastewater during the treatment process. Ampicillin resistance can be attributed to the natural accumulation of the antibiotic in wastewater through agricultural and industrial factors. The minuscule resistance to kanamycin, however, demonstrates the elimination of even ampicillin-resistant coliforms and *E. coli.*

Keywords: *Escherichia coli, coliforms, ultraviolet disinfection, wastewater treatment, ampicillin, kanamycin, antibiotic resistance*

INTRODUCTION

The pharmaceutical industry has long pioneered antibiotic usage to improve the well-being and quality of life of humans worldwide. Unfortunately, the disposal of leftover or unused antibiotics into municipal wastewater, combined with the runoff from agricultural waste, has driven the current antibiotic resistance crisis which continues to be one of the biggest issues in public health (Yu 2022). Yu noted that antibiotic resistance can be caused by a combination of both intrinsic factors, i.e., the development of resistance without gene mutation, and extrinsic factors, which are developed with genetic determinants. These factors create a nutrient-rich environment for antibiotic-resistant bacteria growth and the potential for resistance development through biofilm formation in sewage networks (Tiwari et al., 2022). Municipal wastewater treatment facilities (WWTFs) combat potentially pathogenic microorganisms with disinfection technology, ultraviolet disinfection being a commonly preferred method.

Ultraviolet (UV) disinfection applies electromagnetic energy, typically 250 to 270 nanometers, to the organism through a mercury arc lamp, in which the radiation will denature the organism's genetic material and prevent DNA replication (EPA, 1999). The EPA (U.S. Environmental Protection Agency) notes that the effectiveness of ultraviolet disinfection varies depending on the wastewater contents, the intensity and time of light exposure, and the configuration of the reactor. UV disinfection has shown promise as a treatment of pathogens, due to the ability to set reactor configuration, non-toxicity, and overall disinfection effectiveness. UV technology does face certain obstacles, like economic cost and finding the precise wavelength that most efficiently destroys the microorganisms of interest (Heliyon 2022). Earlier studies have shown that UV disinfection with a selected wavelength destroys or inactivates the genetic material, increasing the overall effectiveness of the disinfection, and with the configuration of the disinfection unit, Wastewater Treatment Facilities (WWTFs) achieve higher inactivation rates of microorganisms, one of the most common being *Escherichia coli* (Kamel 2022).

Escherichia coli, or *E. coli*, is a gram-negative *Enterobacteriaceae* that is naturally occurring in human gut microbiota. Most strains are nonpathogenic, but some may develop resistance and play a role in intestinal infections or diseases (Cabral 2010). Cabral (2010) mentions that *E. coli* is a subspecies of total coliforms, which are gram-negative rods that ferment lactose. Besides Escherichia, coliforms also include the genera *Klebsiella, Enterobacter,* and *Citrobacter.* Coliform abundance is the subject of surveillance to evaluate both microbial quality and treatment efficacy in wastewater, meaning that coliforms like *E. coli* can be used to figure out how effective the WWTF was at disinfecting and inactivating potentially pathogenic organisms and the presence of any coliforms that are resistant to the treatment process (Anastasi 2012). Antibiotics, such as ampicillin and kanamycin, may

be used to eradicate environmental pathogens not previously deactivated from the treatment process.

Ampicillin (AMP) is a semi-synthetic β-lactam antibiotic commonly used to treat *E. coli* infection in humans and livestock; the antibiotic works to inhibit cell wall synthesis of the organism, prohibiting the replication stage from occurring (Li 2019). Recent research has shown that genes in *E. coli* are beginning to show resistance to β-lactam antibiotics, typically by encoding β-lactamases or changing the cell wall target protein, supporting the need for future research on resistance development (Poirel et al., 2018). Kanamycin (KAN) is an aminoglycoside antibiotic that inhibits *E. coli* by blocking the translocation process that produces peptides and is currently an effective antibiotic to treat infections (Liu 2020). Compared to β-lactam antibiotics, aminoglycosides directly target ribosomes and interfere with protein synthesis, damaging the DNA bases of the organism (Kang 2012). My research will study the efficacy of ultraviolet disinfection, found within the local WWTF, and the resistance of E. coli isolates towards two common antibiotics to treat infection: ampicillin and kanamycin. This study will explore UV efficacy and antibiotic resistance in central Kansas and offer an opportunity for further research.

MATERIALS AND METHODS

The purpose of this study is to compare the effectiveness of ampicillin and kanamycin against *E. coli*. The study will be conducted through Turkey Creek, a stream integrated within McPherson, KS. Wastewater samples were collected from four locations, two outside of the local wastewater treatment plant, and two within the treatment process. This treatment facility treats two million gallons of influent wastewater a day using Sequencing Batch Reactor (SBR) technology, a modification of the activated sludge process, and is controlled by a computer and programmable logic controllers (PLCs) that control the influent (raw wastewater), sludge handling, and effluent reuse. Influent wastewater is dispensed into two drum screening channels, two grit removal tanks, and a four-pump lift station that separates the sludge and water. Sludge is further dewatered and transported to an off-site facility for agricultural application, and the effluent is reaerated and disinfected with ultraviolet light before discharge and reuse (www.mcpcity.com).

Four 50mL samples were collected through Turkey Creek: upstream 0.5 miles before treatment, within the WWTF, both pre- and post-UV disinfection, and downstream 0.5 miles after treatment. Samples were collected once a week, every week from September 12th through October 31st, 2023, and physical characteristics of the samples were also taken, including oxygen content, pH, sample temperature, and conductivity using NeuLog Logger Sensors. The sampling locations are specified, and samples were analyzed shortly after collection and 24 hours following (Figures 1 and 2).

Figure 1. Off-site sampling locations.

Figure 2. On-site sampling locations.

Two antibiotics were used to test the antibiotic resistance of wastewater: ampicillin (AMP) and kanamycin (KAN). Ampicillin targets *E. coli* infections, but earlier studies have shown that *E. coli* is beginning to develop resistance against b-lactam antibiotics including ampicillin (Poirel 2018). Ampicillin is an ample subject for antibiotic testing in this stream, especially where agricultural and manufacturing settings heavily contribute to antibiotic disposal and runoff. Kanamycin is used to treat many bacterial infections, and antibiotic resistance is not as common compared to ampicillin. Both treatment groups are compared to a control group treated without an antibiotic, and both coliform and *E. coli* quantification is possible using CompactDry E. Coli (EC) plates.

After samples were collected into 50mL plastic centrifuge tubes, samples were dispensed into one of three glass test tubes treated with ampicillin, kanamycin, or no antibiotic, and each treatment group had two serial dilutions to quantify both coliforms and E. coli, respectively. Once sample

solutions with treatments were prepared, 1mL of the solution was then dispensed into a correlating CompactDry EC plate and stored in a 37°C incubator for 24 hours. Once incubation is complete, colony abundance is then visually counted and recorded. EC plates have two enzyme substrates resulting in coliform colonies appearing pink or purple, and E. coli colonies appearing blue (Hardy Diagnostics). All plates and equipment were autoclaved at 121°C for 35 minutes to ensure proper handling and disposal.

RESULTS

Ampicillin was not shown to significantly reduce the colony abundance, with an average reduction rate of 21.7% in coliforms and 64.9% in *E. coli.* Kanamycin, however, displayed a significant reduction of colonies, with an average reduction rate of 99.4% in coliform colonies and 99.8% in *E. coli* colonies (Fig. 3 and 4). These figures also show that coliforms and *E. coli* both display a moderate resistance to ampicillin, but slight to no resistance to kanamycin.

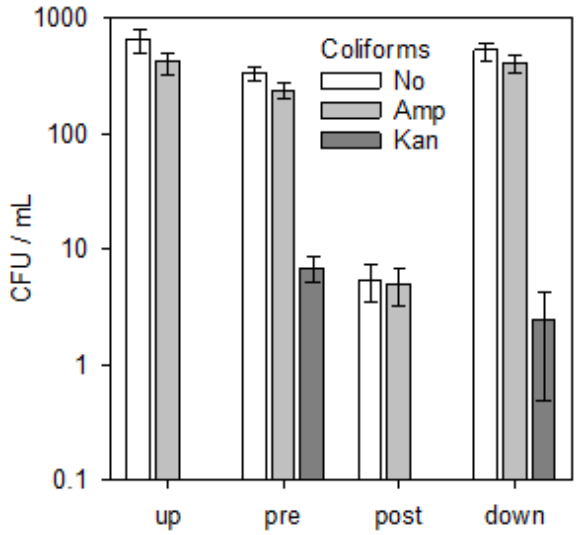

Figure 3. Abundance of coliforms when exposed to different antibiotic treatments.

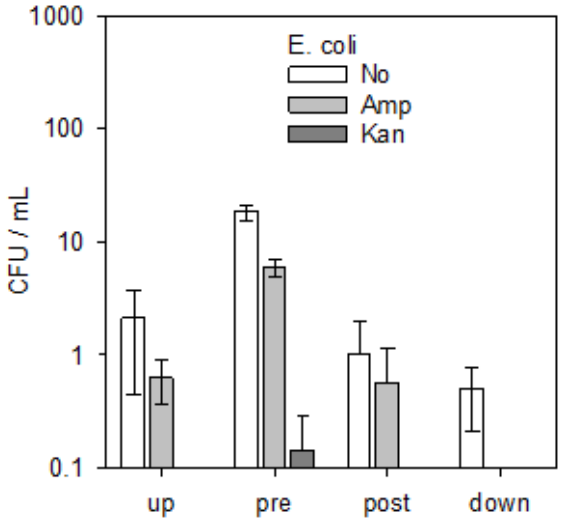

Figure 4. Abundance of *E. coli* when exposed to different antibiotic treatments.

Regarding treatment efficacy, a nearly two-fold reduction in coliforms and one-fold in *E. coli* was observed after UV disinfection (Fig. 3 and 4).

DISCUSSION

Antibiotic resistance is prevalent with antibiotic usage, especially where pharmaceutical waste and agricultural runoff both contribute, and this study shows not only the effectiveness of UV disinfection and each antibiotic but also the resistance of *E. coli* and coliforms against each antibiotic. Ampicillin is more abundant in the environment than kanamycin and is used more frequently in other applications, which supports the study finding that *E. coli* developed moderate resistance against ampicillin. Kanamycin, however, is not as commonly used in the environment and can support the abundance of kanamycin-resistant *E. coli* being low. When presented with an *E. coli* infection, treatment may be considered using kanamycin rather than ampicillin, considering the environmentally developed resistance of each antibiotic found in the study.

Ultraviolet disinfection was also shown to have significantly contributed to the reduction of coliforms and *E. coli* after treatment, leaving very few colonies to have survived the treatment process. The rise in coliforms and E. coli downstream can be associated with wastewater reaccumulating colonies from the natural environment after discharge. Ultraviolet disinfection is found to have eliminated both coliform and *E. coli* strains from the environment, and antibiotics not as commonly used, like kanamycin, can be used to eliminate colonies resistant to other antibiotics. Effective wastewater treatment can eliminate even antibiotic-resistant bacteria, and the presence of colonies after disinfection or antibiotic treatment can support the emergence of antibiotic-

resistance research. Further study could examine the microbial load shortly after pharmaceutical disposal and agricultural runoff to determine how much each contributes to antibiotic resistance. Regarding antibiotic efficacy particularly, further study can expand on what antibiotics are best to treat environmental bacteria, like coliforms and *E. coli.*

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Research Awards in the Natural Sciences

The awards are sponsored by the Natural Sciences of McPherson College and Midwest Oilseeds of Adel, Iowa.

The **Burkholder Research Award**, the highest award, is presented in recognition of outstanding achievement in student research. The **Merit Research Award** is presented in recognition of achievement in student research.

Each student completing a senior research project in the Natural Sciences is a candidate for an award. The Natural Science Faculty select the winners of the awards. Three criteria are used to judge the quality of the research and in selection of student award winners: (1) Selection and planning of a research project; (2) Quality of the research work, including techniques, observations made, and the analysis of data; and (3) Reporting the research, consisting of preparation of the research paper, and a poster or oral presentation to students and faculty.

Each student receiving an award will receive a Certificate of Award. Those receiving the Burkholder Award will have their name inscribed on a plaque, and will receive a year membership in the American Association for the Advancement of Science and a subscription to the journal *Science*.

Year	Burkholder Award	Merit Award
2024		Taylor Bohannon, Maggie Brown, Madison Turley
2023	Olivia Smith	Bethany Masters Ice, Nathan Saffer, Ann Marie Weesner
2022		Brionnah Fessler, Erica Paradise, Emma Singleton
2021		Sydney Burton, Jessica James, Choucranie N. Kayembe, Aaron Leck, Allison Pamela Penalva Vanegas
2020		Sebia Kalambayi Kabedi, Garrett Owen
2019	Parkes Wolters	Micaila Curtis, Samantha Nelson
2018		Nora Grosbach, Amy Makovec
2017	Nathan Finch	Sheryl Evans, Lucas Giesey
2016	Tiffany Fraser, Ashley Long	Alia Khalidi, Kaley Kinnamon
2015	Nathaniel Schowengerdt	Shannon Coldren, Sydney Lipton, Jordan Stewart
2014	Yi Qun Chai, Sean DeYoung	Lori Crain, Alejandro Esparza, Christian Rodriguez
2013	Amanda Baxter, Emily James, Taylor Roop	Torey Fry, Kasey Miller
2012	Audrey McTaggart	Savannah Sievers, Andrew Skinner
2011		Karissa Ferrell, Kelley Green, Ashley Zodrow
2010	Ashlee Jost, David Miller	
2009	Adam Horinek	Amanda Pangburn, Nicole Sampson, Lezli Warkentin
2008	Joel Grosbach, Landon Snell, W. Brett Whitenack	Alan Grosbach
2007	Callie Crist	Rhonda Hoffert, Jamie Rodriguez
2006	Travis Allen	Lisa Sader
2005	Joseph Blas	David Cockriel, Jenny Harper, Danielle Lucore
2004	Robert Ullom	
2003	Michelle Schulz	Adeline Cripe
2002	Elizabeth Stover	Renata Lichty
2001	Genelle Wine	Jonas Lichty
2000	Nathan McLaughlin	Jeffrey McPherson
1999	Roy Johnson, Jr.	Jennifer M. Amiot, Janet Bowen, Eric D. Putnam, Anna Katharina Schenk
1998		Rebecca Standafer, Cameron Mahler
1997	Kerri Kobbeman	Rod Samuelson
1996		Chris Owens, Wes Seckler, Stasi Zirkel
1995	Monica Embers, Heather Hughbanks	Erik Harmon
1994	Adam Smith	Susan Blubaugh, Sherry Coopple, Adeola Grillo, Paula Worley

1993	Tyson Burden	Robin Morgan
1992	Pete Hanson	
1991		Thomas Champion, Shannon Hull
1990	James Dechand	David Maxey
1989		Michelle Roesch
1988	David Lehmen	Cynthia Aeschacher, Sandra Ashbaugh
1987	David Krehbiel, Marla Ullom	Cassandra Clark, Marsha Morley, Jay Nicholson

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