A Study On The Short-Term Effects of The Antioxidants Vitamin C and Vitamin E on the Human Immune System

Research Question: How do different doses of the antioxidants Vitamin C and Vitamin E affect the human immune system in the short-term?

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Chapter One: Introduction

1.1 Immune system

The immune system helps protect and defend the host's body from harmful substances such as pathogens, which triggers the recognition of self, and the response to nonself (Schultz and Grieder, 1987). One of the types of immunity includes innate or nonspecific, which serves as the first line of defense in barricading harmful substances from entering the human body (Response, 2017). The epithelial surfaces, which includes the skin, acts as the first barrier which is impermeable to most antigens. This consists of shedding of the layer of dead skin, meaning there is a constant removal of bacteria or other infectious agents that have clung onto that layer. In addition, within the respiratory and gastrointestinal tract, mucus that lines the tract helps trap the agents from entering the lungs or the digestive system. The tears, saliva, and nasal secretion include chemical factors such as lysozyme and phospholipase, which help breakdown the cell wall of bacteria, thereby killing it (Microbiologybook.org, 2017).

When bacteria establish themselves in the underlying tissue and causes damage to surrounding cells, phagocytic white blood cells, such as macrophages, recognizes the pathogen as 'nonself' from their surface proteins, antigens. The macrophages then engulf and partially digest the pathogen through a process known as phagocytosis, and present the pathogens antigen on their surface. If this process does not stop infection, lymphocytes, a type of white blood cell that overcompasses the immune system comes into play. T-lymphocytes (T-cells) then chemically recognize the antigens on the macrophage and become activated, turning the nonspecific response to a specific one. When an antigen gets past the nonspecific defense, T-cells attack the antigens directly, while dividing rapidly and releasing a chemical, known as

cytokines, to coordinate the immune response (such as stimulate new T-cells or attract more macrophages) (response, 2017). The T-cells divided are either Memory T-cells that stay within the blood plasma, or Helper T-cells that travel to the lymph nodes to activate B-lymphocytes (B-cells). Similarly, activated B-cells divide rapidly to form two types of cloned cells; plasma cells that secrete antibodies immediately to fight the infection, or memory cells that circulate in the bloodstream waiting for a secondary infection.

Antibodies help phagocytes bind to a specific antigen, making it easier and faster for the immune cells to destroy the antigen. This can be done either through signalling to other cells for help, or through the process of agglutination, which consists of binding the antigens together to mark them for destruction. An example of the antibodies found in the human immune system is Immunoglobulin G (IgG), which is found in all body fluids, thus being the most abundant type of antibody. Another example is the Immunoglobulin M (IgM) which is the "first antibody to be made by the body to fight a new infection", and is found mostly in the blood and lymph fluid (Kidshealth.org, 2017).

1.2 Antioxidants and Vitamins

The immune system consists not only of lymphocytes, but also antioxidants, including multiple vitamins, such as the common ascorbic acid (Vitamin C) or Vitamin E (Brambilla et al., 2008). Antioxidants are commonly used under the assumption to promote optimal health, as they are known to combat aging, as well as produce anti-apoptotic and immunostimulatory effects (Goswami et al., 2013). These antioxidants help prevent the attack of free radicals on the immune system, which are compounds that consist of an unpaired electron in the outer orbital, making them highly reactive. A subgroup of free radicals are known as reactive oxygen species

(ROS), which are caused by how oxygen consists of two lone pairs, thus making it very likely to form free radicals. Examples of ROS consists of molecules such as the hydroxyl radical and hydrogen peroxide, which damage proteins, DNA, and lipids, which in turn destroys the body's cells and tissues. An additional role of the antioxidants includes their ability to transform the ROS into harmless compounds, through a redox-based mechanism. However, when the balance between the ROS and antioxidants are disrupted, or in other words when the body is unable to defend against their harmful effects, a condition known as oxidative stress arises, which has been linked to several diseases (Brambilla et al., 2008).

Vitamins are divided into two different types - water-soluble vitamins and fat-soluble vitamins. This classification depends on how the human body dissolves and stores these vitamins. The former dissolves in water and generally are not stored in the body, while the latter is stored in the fatty tissues and liver, and are used whenever the body needs it (WebMD,2017). Currently, there has been an assumption of how over consumption of vitamins have been linked to negative effects such as cancer, instead of producing beneficial effects (Brambilla et al., 2008). This stems from how nowadays, there has been the problem of over-supplementation and self medication of antioxidants, which adds on to the controversy of whether or not there is a significant effect on the human body's immune and defense system (Field, Johnson and Schley, 2002). There is an uncommon opinion of how intaking several antioxidants and other drugs may lead to harmful side effects (Brambilla et al., 2008). Many people seem to look over the fact of how sufficient levels of antioxidants can be obtained directly from the daily diet. In fact, a diet rich in antioxidants has been shown to correlate directly with decrease in cancer, especially

observed in the elderly, while in younger individuals an increase in cell-mediated immune responses was seen (Hughes, 1999).

A common antioxidant known as Vitamin C, or ascorbic acid, acts as a physiological antioxidant in the immune system, protecting against oxidative stress caused by infectious pathogens. Vitamin C is an example of a water-soluble vitamin, meaning they continuous daily intake for the body is required (WebMD, 2017). A result of Vitamin C intake has been correlated with high concentrations of phagocytes and lymphocytes, indicating the role Vitamin C plays in the immune system (Hemilia and Louhiala, 2007). Currently, Vitamin C is prescribed or unnecessarily taken during antibiotic therapy or bacterial infection, leading to bacterial resistance and continual progression of a patient's disease (Goswami et al., 2013).

This has lead to the controversy between the effects of Vitamin C on the host's immune system, as there has been studies that suggest both sides of the argument. Multiple studies suggests that high intakes of Vitamin C along with other antioxidants are linked to lowered risks of chronic diseases (Field, Johnson and Schley, 2002), as it helps increase "the functioning of phagocytes, the proliferation of T-lymphocytes, and the production of interferon, and decreased replication of viruses," (Hemila and Louhiala, 2007). However, current studies have shown conflicting results, such as how excess amounts of Vitamin C can cause kidney stones (WebMD, 2017).

Not only does Vitamin C play a role solely in the immune response, it is also involved with an additional antioxidant, Vitamin E, as it helps regenerate the antioxidant from its oxidized form (Linus Pauling Institute, 2017). In addition, Vitamin C can help reduce damages made by ROS to lymphocytes through the regeneration of Vitamin E (Field, Johnson and Schley

2002).Vitamin E's main job in the immune system is protecting the integrity of cell membranes from free radicals, similar to all other antioxidants, which is once again dependent on the status of Vitamin C to a certain extent. Other than being involved with Vitamin C, a particular type of Vitamin E, the a-tocopherol form of this antioxidant helps prevent peroxidation, or the degradation of polyunsaturated fatty acids, a molecule relevant to our body. If the peroxidation were to happen, cellular damage and eventually improper immune systems will follow, thus implying the relevance of Vitamin E (Linus Pauling Institute, 2017).

Similar to Vitamin C, there is a current over supplementation of Vitamin E, which has accordingly "enhance immunity and decrease susceptibility to certain infections", with the target group being elderly individuals (Linus Pauling Institute, 2017). Contrastingly, Vitamin E is an example of a fat-soluble vitamin, meaning accumulation of excessive Vitamin E can lead to a condition known as hypervitaminosis, or excessive amounts of vitamin, and can increase the risks of hemorrhaging, the rupturing of blood vessels (WebMD,2017). On the other hand, an additional study has shown how high doses of Vitamin E has seemingly not result in any damage in peripheral blood lymphocytes, and is an antioxidant that is considered safe even when consuming high doses. Consuming high doses of Vitamin E for a long period of time, however, has been connected to inhibiting certain areas of the immune response, indicating there may be a specific limit for this antioxidant (Field, Johnson and Schley 2002).

Chapter 2: Methods of Investigation

Due to limited time and equipment, secondary research is required to be able to answer the research question. Data will be extracted and manipulated from different sources, with the investigations concerning the dosage of Vitamin C and Vitamin E and how it affects the host's immune system. This includes results published by reputable journals, to articles published by other biologists. In addition, the different sources had conducted their experiments on animals of different species: cats, broilers, roosters, and humans; so it is important to note that the underlying assumption is made that the human immune system and the investigated immune systems are relatively the same. Given the fact that the effects of drugs including antioxidants are globally tested on animals, results manipulated within this investigation should be deemed reliable.

The independent variable of this investigation will be the different doses of the antioxidants under study, with the intervals being determined by the recurring doses of each antioxidant whilst during research. The doses are: Control (0 mg/kg), Low (between 10 and 50 mg/kg), Medium (100 mg/kg), and High (200 mg/kg or higher). The dependent variable is not fixed, but is the broader discussion of the short-term effects of each antioxidant on the host's immune system, with main focus on the lymphocytic populations. From the trends observed, the optimal dose of each antioxidant will then be determined, based upon the fact that optimal means the most effective immune system within the range of the doses. Furthermore, as most of the data sources lacked uncertainties within their results, the manipulated data itself had no uncertainties, which will be discussed in the evaluation section.

Controls within this investigation includes the range of each dose of antioxidants; this was crucial to maintain in order to be able to correctly identify the short-term effects of each dose on the immune system and select the most optimal dose. Additionally, the length of time each experiment took place should be around four weeks, as this provides the immune system the same amount of time to produce a response, which will then lead to reliable conclusions. As a means to make the data original, the t-test was used between the doses (which were used as 0, 30, 100, and 200 mg/kg) and the observed immune response. The t-test should help provide greater insight into the significance of the data and the reliability of the conclusions drawn. T-test values with a p-value smaller than 0.05 are viewed as statistically significant, meaning that the conclusions draw are more reliable and are less likely to have occurred by chance. With this being the case, the hypotheses are:

Null Hypothesis: Different doses of Vitamin C or Vitamin E does *not* result in a statistically significant difference on the human immune system.

Alternative Hypothesis: Different doses of Vitamin C or Vitamin E does result in a statistically significant difference on the human immune system.

Chapter 3: Data Analysis (Collection and Processing)

3.1 Raw Data for Vitamin C

Table 1: Table showing the varying doses of Vitamin C, from the control up to 200 ppm, and the effects on lymphocytic cells for broilers' immune system after four weeks. The p-values are also indicated here, signifying if there is a statistically significant relationship between the two variables (Lohakere et al., 2005)

	Control	Vitamin C (ppm)					*P*-value
	0	10	50	100	200	SE[1]	Linear
Lymphocytic cells							
MHC-II	6.38	10.7	7.00	5.28	8.50	3.98	NS[2]
CD4[3]	21.53^{c}	33.08^{bc}	28.43^{bc}	58.43^{a}	41.53^{b}	12.03	0.0059
CD8	6.73	5.38	11.48	8.08	14.75	5.34	NS
TCR-I	14.45	12.13	17.6	10.75	12.68	3.77	NS
TCR-II[3]	17.95^{b}	15.20^{b}	22.30^{ab}	33.40^{a}	27.00^{ab}	8.07	0.0421
B-cells	2.93	2.03	3.43	3.73	1.50	1.62	NS

This table displays a correlation of increasing doses of Vitamin C and how there is a general increase in most of the lymphocyte subpopulations with optimal responses in 100 ppm, with some of the immune responses having a P-value smaller than 0.05, signifying a statistically significant relationship.

Table 2: Table displaying the varying doses of Vitamin C, from the control up to 200 ppm, and the effects on plasma and liver vitamin content (ppm) in broilers, along with the P-value (Lohakere et al., 2005).

	Control	Vitamin C (ppm)					*P*-value	
	0	10	50	100	200	SE[1]	Linear	Quadratic
Plasma (ppm)	7.46^{e}	10.71^{d}	13.56^{b}	13.71^{a}	13.49^{c}	2.72	0.0001	0.0001
Liver (ppm)	28.83^{c}	51.34^{b}	53.54^{a}	53.45^{a}	53.45^{a}	10.83	0.0001	0.0001

This table displays the effect of Vitamin C on the plasma and liver content, with a significant increase in the liver content as the doses of Vitamin C are increased. It seems as though there is a plateau when the Vitamin C doses are higher, as the numbers of both the plasma and liver vitamin content in the 100 and 200 ppm doses are relatively the same.

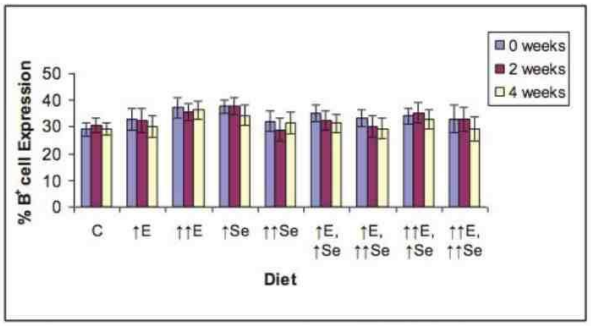
Table 3: Table showing the levels of Vitamin C, the placebo group, or low, medium, or high levels in the plasma, and the effects on the mean duration of colds, measured in days. (Vitamin C and Infectious Diseases, 2017).

Vitamin C level in plasma	Episodes (no.)	Mean duration (days)	Difference from low-placebo
	Placebo group		
Low	20	5.6	0%
Middle	18	4.5	−20%
High	10	4.4	−21%
	Vitamin C group (1 g/day)		
Low	22	4.0	−29%
Middle	15	2.7	−52%
High	13	6.8	+21%

This data supports the research mentioned in the introduction, of how Vitamin C helps cure the cold (Field, Johnson and Schley, 2002). Focusing on the Vitamin C group, it seems as though the result of higher levels of Vitamin C helps cure is correct, but only if there is an intake of middle doses of Vitamin C, as high doses have resulted a longer period of time for recovery.

3.2 Raw Data for Vitamin E

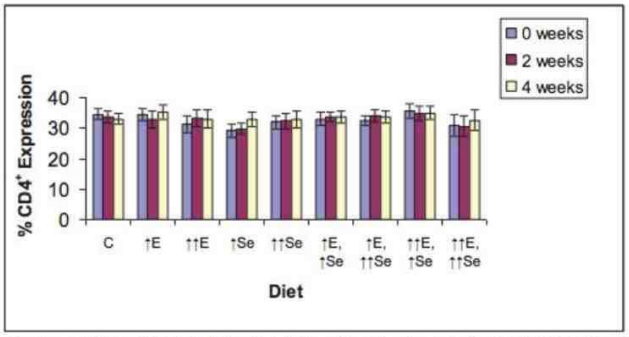
Table 4: This figure is depicting the $\%B^{+}$ cell expression throughout the duration of the feline diet, four weeks, with emphasis on varying the amounts of Vitamin E (O'Brien, 2010).



Diet Key: C= Control; ↑↑E= HVitE (500 IU/kg diet); ↑↑Se= HSe (10 mg/kg diet); ↑E= MVitE (250 IU/kg diet); ↑Se= MSe (2 mg/kg diet) (n=8)

The main focus is on the first three group of columns, as these represent the control, low and medium doses of Vitamin E, and is where the high dose is manipulated from. Looking from the figure, there seems to be an optimal level of B cells in the 0 weeks for all doses, with slightly varying data points after two weeks and four weeks.

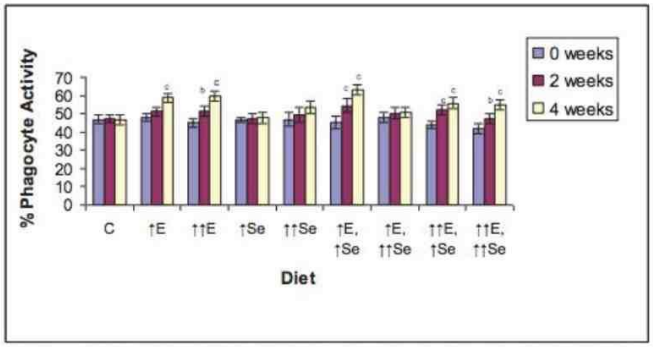
Table 5: This figure shows the CD4+ cell expression similar to the figure above, throughout the duration of the feline diet, four weeks, with emphasis on varying the amounts of Vitamin E (O'Brien, 2010).



Diet Key: C= Control; ↑↑E= HVitE (500 IU/kg diet); ↑↑Se= HSe (10 mg/kg diet); ↑E= MVitE (250 IU/kg diet); ↑Se= MSe (2 mg/kg diet) (n=8)

The main focus is on the first three group of columns, as these represent the control, low and medium doses of Vitamin E, and is where the high dose is manipulated from. As seen in the figure, there seems to be an optimal level of CD4-cells during the zero week of the diet, with slightly varying percentage of expression between the following four weeks.

Table 6: This figure shows the phagocytic activity (%) throughout the duration of the feline diet, four weeks with variation in the amounts of Vitamin E (O'Brien, 2010).



Diet Key: C= Control; ↑↑E= HVitE (500 IU/kg diet); ↑↑Se= HSe (10 mg/kg diet); ↑E= MVitE (250 IU/kg diet); ↑Se= MSe (2 mg/kg diet) [a] p<0.05;[b] p<0.01;[c] p<0.001 (n=8)

The main focus is on the first three group of columns, as these represent the control, low and medium doses of Vitamin E, and is where the high dose is manipulated from. From the figure, there seems to be the highest phagocytic activity in the medium dose and after four weeks.

Table 7: This table shows a direct primary immune response of broilers, depending on the dosage of Vitamin E (mg/kg) and the effect on the titers of total antibodies (Niu et al., 2009) .

Treatment	Primary		
	Total antibody	IgM	IgG
Vitamin E (mg/kg)			
0	3.56^{c}	2.48^{b}	1.29^{b}
100	4.89^{b}	3.13^{ab}	1.85^{ab}
200	5.82^{a}	3.61^{a}	2.01^{a}

The data extracted is from the primary response, with consideration of the total antibody for the three doses of Vitamin E given in their diet. There is an obvious increase in the total titres of antibody of the broilers in all of the doses of Vitamin E, meaning the 200 (mg/kg) dosage had the highest number of antibodies.

Table 8: This table shows the varying doses of Vitamin E (mg/kg) and the effect on the amount of macrophages (%) in broilers, calculated from approximately 300 adherent cells per coverslip (Nieu et al., 2009) .

Vitamin E (mg/kg)	Temperature	AEC[1] ($\times 10^{6}$)	Macrophages[2] (%)
0		5.50^{b}	92.40^{b}
100		7.10^{ab}	93.05^{ab}
200		7.37^{a}	94.21^{a}

This data focuses merely on the percentage given for each of the doses of Vitamin E in the broiler's diet. Once again, there is an increase in macrophage activity as the dose of Vitamin E increases, signifying how this antioxidant has improved the immune response.

3.3 Qualitative Data

Whilst collecting the data, it was noticed that all the lymphocytic populations were affected within the immune response, no matter how much. Although uncertainties within each experiment were not given exactly, it was also noted that Vitamin C data was easier to find than Vitamin E data, showing the differences in the amount of research as Vitamin C is the more bought antioxidants. Before processing the data, it was observed how different doses of each antioxidant do seem to make a difference within the rate of the immune response, and there is always an increase in the immune system's response compared to the control dose.

3.4 Processed Data

Table 9: Table displaying the processed data of Vitamin C; how the different doses of Vitamin C (mg/kg) has affected the immune system; mostly concentrated on lymphocyte subpopulations, along with the t-test values.

Doses of Vitamin C (mg/kg)	Liver Vitamin Content (ppm)	T-cell Receptor II Population	Mean Duration of Colds (days)	CD4-Cell Population	B-Cell Population
Control	28.83	17.95	5.6	21.53	2.93
Low	52.44	18.75	4.0	30.76	2.73
Medium	53.45	33.40	2.7	58.43	3.73
High	55.16	27.00	6.8	41.53	1.50
T-Test (p=...)	0.18	0.11	0.06	0.15	0.06

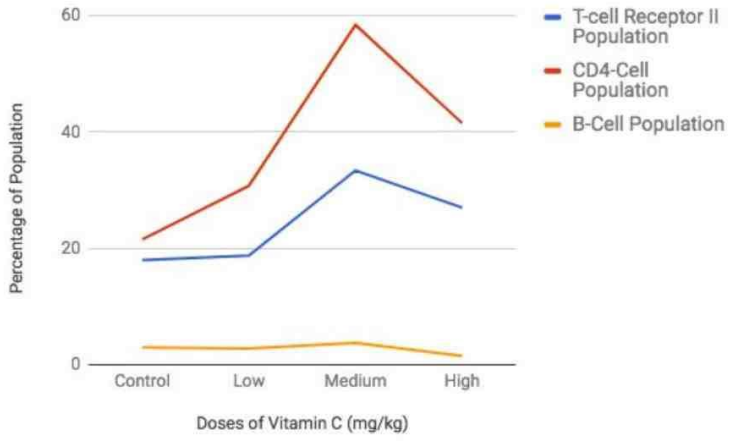
This table shows how different amounts of Vitamin C proves to improve the immune system, by increasing certain subpopulations of specific cells. Although most values increases from its initial value of the control, with the exception of the mean duration of colds that seems to lack correlation, each type of cell seems to have an optimal amount at the medium doses, although the t-test values does not prove to be statistically significant, since $p>0.05$.

Table 10: Table exploring the processed data of Vitamin E, as well as the different parts of the immune system affected, with a main focus on the lymphocytic subpopulations, along with the t-test values.

Doses of Vitamin E (mg/kg)	Phagocytic Activity (%)	Macrophages (% per 300 adherent cells per coverslip)	Titers of Antibodies (First Response)	% $CD4^+$ Expression	%B^+ Cell Expression
Control	47.0	92.40	3.56	33.0	31.0
Low	51.7	92.69	4.28	32.0	33.0
Medium	52.3	93.05	4.89	32.5	36.0
High	52.9	94.21	5.82	33.1	37.0
T-Test (p=...)	0.21	0.45	0.05	0.12	0.13

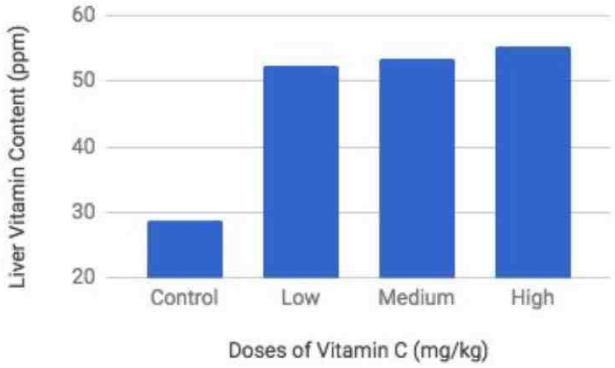
There seems to be no significant improvement in the immune system when increasing the doses of Vitamin E. Most of the data points are higher when compared to the control experiment, showing how Vitamin E does affect the immune system. The t-test values does not prove to be statistically significant, for each part of the immune system except for amount of antibodies which had the closest p-value of p=0.05.

Figure 1: This figure displays information from Table 9; effects of different doses of Vitamin C (mg/kg) on the x-axis, and on the percentage of lymphocytic populations, on the y-axis.



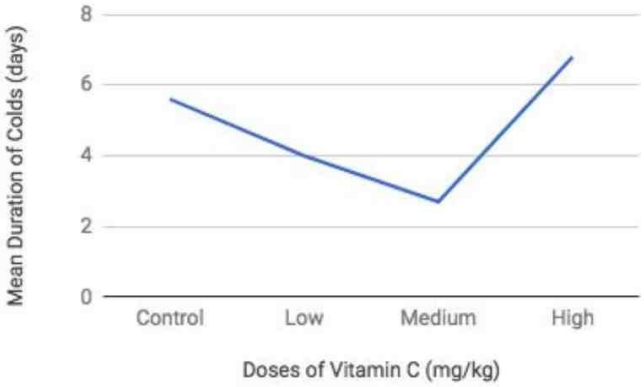
It seems as though the optimal dosage of Vitamin C seems to be the medium dosage, as the majority of the immune response is high there before dropping down in the high dose. Each type of cell has an increase in population after the Control experiment, showing how intake of Vitamin C does affect the immune system.

Figure 2: Figure from data extracted from Table 9 showing the effects of different doses of Vitamin C (mg/kg) on the x-axis, on the liver vitamin content (ppm), on the y-axis.



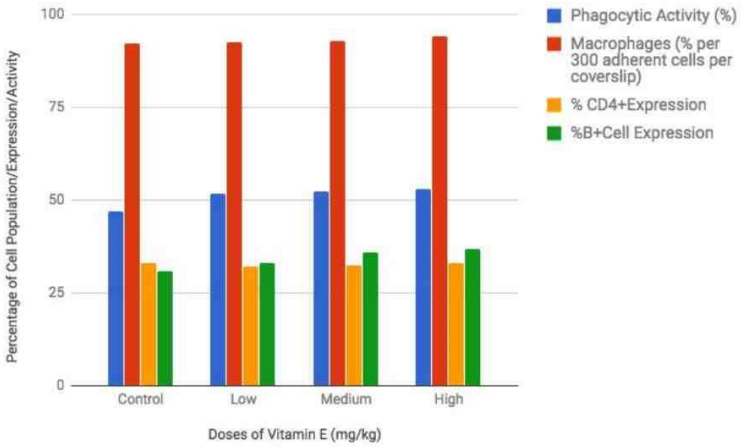
The liver vitamin content seems to be the highest at the high dose of Vitamin C, although there is a large spike from the control dose to the low dose. From there, a gradual increase in the liver vitamin content is seen; reaching the highest at 55.16 ppm.

Figure 3: Figure from data extracted from Table 9 showing the effects of different doses of Vitamin C (mg/kg) on the x-axis, on the mean duration of colds (days), on the y-axis.



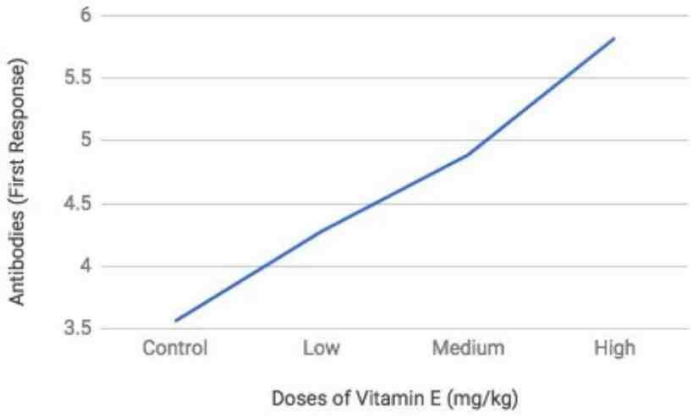
Similar to previous figures, there seems to be an optimal response at the medium dose, as the duration of colds is the shortest. After the medium dose, however, high amount of Vitamin C seems to increase the duration of colds to a week, longer than not consuming any at all.

Figure 4: Figure displaying most of the values presented in Table 10, with the effect of different doses of Vitamin E (mg/kg) on the x-axis, on the percentage of the different lymphocytic cell expressions or activity on the y-axis



For each of the lymphocytic populations or activity, there seems to be a gradual increase in the cell expression or population or activity from the control experiment, meaning the high dose had the optimal response for all lymphocytic cells.

Figure 5: Figure displaying the rest of the values presented in Table 10, with the effect of different doses of Vitamin E (mg/kg) on the x-axis, on the titers of total antibodies in the first response, on the y-axis.



The total titers of the antibodies found within broilers shows an increase from the control dose to the high dose, conveying how increasing in Vitamin E dose increases the response of the immune system.

3.5 Sample Calculations

T-Test Values (Google Spreadsheet) - (Table 9 - Titers of Antibodies First Response)

The A3:A6 is the dose of Vitamin E entered, with the D3:D6 being the antibodies, the 1 representing a one-tailed test, and the 2 representing an equal variance test.

Graphs: all graphs were produced by Google Spreadsheets, with the doses of each antioxidant in one column with the observed variables in another column.

2.3 Results and Interpretation

From the processed data, there seems to be an optimal increase in the immune system for Vitamin C at the medium dose, as shown in Figure 1 from the spike in lymphocytic populations, to Figure 3 where the mean duration of colds is the shortest. At the high dose in both of these figures, there is a reverse effect on the immune system; the lymphocytic populations drop significantly, while the mean number of colds increases to more than the control trial. For the effect of Vitamin C on the liver vitamin content (ppm), however, high doses of Vitamin C revealed highest liver vitamin content. For all of the Vitamin C data, there seems to be no statistically significance when looking at increases doses of Vitamin C on the immune system, as all p-values were larger than 0.05 as seen in Table 9.

On the other hand, looking at Vitamin E, there seems to be a gradual increase in the percentage of cell expressions and cell activity as the dose of Vitamin E increases, as seen in Figure 4. Despite the slight dip between the low and medium dose for the CD4-Cell expression, there is an upward trend from the control dose all the way up to the high dose, signifying how Vitamin E does help boost the immune system. Even in Figure 5, the titers of total antibodies

increases constantly, showing how each specific cell involved in the immune response seems to be affected at varying degrees. Similar to Vitamin C, Vitamin E and the immune system have no statistically significant relationships, as each p-value is larger or equal to 0.05, as seen in Table 10.

Chapter 4: Conclusion

In conclusion, it seems as though increase in doses of Vitamin C helps improve the overall human immune system. Based on both the raw data and processed data, there is a prevalent trend of how additional Vitamin C in the diet does help increase the rate of immune responses for each of the experiments, whether it is a human trial or an animal trial. However, the exact dosage for optimal immune response falls mainly on the medium dosage of Vitamin C, of around 100 mg/kg, as the values obtained clearly indicate optimal behaviour of the immune system. This is even clearer in Figure 1, as there is a peak on the medium dose, compared to other doses that signify only a slight increase in the immune response. This implies that Vitamin C does help combat colds and other pathogens involved, as it helps produce or amplify the specific cells required to combat pathogens, including T-cells, B-cells, or CD4 cells.

Additionally, although the liver vitamin content (ppm) seems to continuously increase as the dose of Vitamin C increases as seen in Figure 2, it does not mean that there is no negative effects on the immune system, as this statistic merely points out how the body stores additional antioxidants and does not take into account the specific populations of each lymphocyte. Moreover, excessive amounts of Vitamin C do seem to negatively affect the immune system as well, as seen in the decline in lymphocytic populations in Figure 1 and the subtle increase in the mean duration of colds in Figure 3. This helps prove that although higher doses of Vitamin C does boost "the functioning of phagocytes" and the "proliferation of T-lymphocytes", there is a limit to the consumption level (Hemila and Louhiala, 2007), thus adding to the reliability of this conclusion.

Having p-values that were all larger than 0.05 results in the accepting of the null hypothesis, which means different doses of Vitamin C does not result in a statistically significant difference on the human immune system. However, as the values were close to the cut-off, as displayed in Table 9, the marginal errors must be considered. This means there could still be a possibility in which the relationship derived occurred due to chance, yet the confirmed results from other sources helps increase the reliability of how Vitamin C doses do help increase the immune response to a certain limit.

Conversely, Vitamin E does also seem to help increase the immune response. As seen in both Figures 4 and 5, increases in Vitamin E doses seem to constantly increase lymphocytic population and activity, including the CD4-cells expression, B-cell expression, macrophages, phagocytic activity, and the titers of total antibodies. The optimal dosage seems to be the high dose of around 200 mg/kg, as unlike Vitamin C, there is no decline in the immune system, which correlates with the findings of Field, Johnson, and Schley 2002, adding reliability to the conclusions drawn.

Despite this, it must be considered that Vitamin E, a fat-soluble vitamin is stored within the liver, meaning accumulation could lead to harmful effects. As this investigation was looking at the short-term effects of around four weeks of Vitamin E on the immune system, long-term harmful effects have not been considered. Similar to Vitamin C, the p-values for Vitamin E were also larger than 0.05, meaning the null hypothesis was accepted, which means different doses of Vitamin E does not result in a statistically significant difference on the human immune system. However, as the calculated p-values in Table 10 included one that was exactly 0.05 and others were near the cutoff range, the reliability of the conclusions drawn is still justified by the

marginal errors that may have occurred. Additionally, the supportive data as mentioned earlier helps indicate that results received are still justified, as the short-term effects of Vitamin E do prove to at least temporarily boost the immune system.

Chapter 5: Evaluation

A strength in this lab included how multiple sources were used in order to obtain the given conclusion. Due to how this was a secondary research lab report, a number of reputable sources were required and used in order to ensure an accurate conclusion was drawn out. This helped increased the reliability and accuracy of the processed data and results, as different experiments may have had different conditions in terms of nurturing the animal whilst conducting a trial, and thus produce different conclusions. However, since the data was extracted and manipulated to answer this investigation, the conclusion drawn is able to be further backed up by the cited sources.

Another strength in this lab included how the data was manipulated instead of being used directly. This is because if the data that was drawn was just used without further interpretation, then the lab will be a repetition of the labs already conducted, providing no further and original insight into the research question. But, as the multiple data sources used consisted of data tables with different values for factors in the immune system, the method that manipulated the data in order to find a trend or pattern helped produced a new set of data that displays the relationship that was found. This helped increase the reliability of the conclusion drawn, as there was no bias in deducting this conclusion, unlike possible biases that might have stemmed from other lab conductors that wanted to back up their own hypothesis or theories.

Conducting a t-test on the processed data was also a strength, as it helped support the conclusion and judged the reliability of the conclusion drawn. Despite most p-values being $p>0.05$ and proving there is a possibility that the conclusions drawn were incorrect and the correlations seen occurred by chance, this helps suggest the errors in the experiment, thus being a

strength in determining the extent of how accurate the conclusions were. Additionally, the p-values that were larger than 0.05 could have stemmed from the lack of consideration of the uncertainties within each experiment, as well as how an average number was used to calculate the t-test values. In other words, when calculating the p-value, the low dose was calculated as 30 mg/kg and the high dose calculated as 200 mg/kg; this means that all experiments that didn't provide the trials with this dose did not have the same p-value.

This points out one of the prominent weakness of this investigation; which is how it is a secondary research lab, as the data used to derive a conclusion was not conducted individually. As a result, there could have been some procedural errors in the experiments itself, but without recognition in finding trends for this lab. In addition, each experiment included different procedures, meaning the uncertainty for each step or for a whole experiment was not able to be completely included or correctly identified into consideration when interpreting the data. This could have affected the results significantly, since if there was any important information or uncertainties in the methodology which affects the data without acknowledgement in this lab, then the trends observed would be altered. Furthermore, an altered trend, which could include a larger range of antioxidant doses or outliers for each part of the immune system could produce a more reliable and different conclusion than the one drawn. A realistic and obvious solution to this would be to conduct the experiment individually, as all uncertainties or procedural errors along with qualitative observations can be taken into account when drawing a conclusion. This solution is under the assumption that there is an extended amount of time given for the lab to be done, as well as sufficient resources and equipment required to collect reliable and sufficient data.

Another weakness includes how the data used in finding a conclusion was derived from experiment that were conducted on animal trials of different species. Even though the experiments that were conducted on humans were scarce, all of the experiments cited were looking for the same conclusion, of how the different doses of Vitamin C and E affected the immune system. However, such said goals of each experiment varied, meaning the circumstances of each experiment could have differed. This could mean that each species of animals were treated differently prior or during the experiment was conducted, which may have factored into affected the immune system differently. As a result, this could have lead to an incomplete or untrue conclusion, as the causation deducted could have been false due to misevaluation. Although a realistic solution to this could be to conduct a primary research, if there are still the same circumstances regarding the limited time and resources, the research question could be phrased differently, to find the effect of different doses of Vitamin C and Vitamin E for a specific type of species, such as broilers, which has an abundant amount of research already conducted. This would mean that the amount of uncertainty and unreliability would be decreased, leading to a relatively more reliable data than the received one.

Lastly, another affecting weakness is how the studied antioxidants included only Vitamin C and Vitamin E. Although this was necessary in order to have a scope on the research question, and chosen due to the global trend, this could have affected the data received, as there could have been a correlation in the immune system that could have caused the results seen, instead of having a direct causation. In other words, varying the doses of the studied nutrient antioxidants has led to the mentioned conclusion, yet other antioxidants could have been affected as a further reaction that could have affected the immune response. In order to solve this, the experiment

could also take into account other antioxidants that were already present in the immune system prior to the experimentation, and see whether other antioxidants were involved in the response noted. This being the case, a further experiment could involve the investigation of the interactions between several of the antioxidants, and how they cumulatively affect the immune response.

Chapter 6: Bibliography

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