Investigating the effect of antibiotics on the fermentation on three different types of milk: Cow, Goat and Buffalo.

INTRODUCTION

Milk is one of the most crucial sources of nutrition for humans, especially new-born babies, as they are still building up and getting accommodate to their immune system. The milk provided by a mother is not only considered necessary for a child's growth but it also one of the main elements needed to build up a robust immune system. As suggested by various doctors, after a child undergoes breastfeeding in his earlier stages, the second step towards building a healthy defense mechanism is exposure to animal milk, precisely that of a cow. However, while keeping in mind the benefits provided by breastfeeding, it is also essential to keep in mind the varied types of milk, such as that of a buffalo and goat, which not only have a high nutritional value of milk [1]but also stay fresh for an extended period. The incident that acted as a stimulus for me to pursue this topic is that once while I was heading for my vacation, I came across a talk between my mom and her friends stating the that goat milk is best as it takes longer time to stale compared with other kinds of milk. I was always curious why babies are proposed to animal milk, why animal milk only? This thought provoked me to do an investigation on milk and antibiotics.

However, babies below or of the age of 12 months cannot be fed with this animal milk because they cannot digest animal milk as quickly as mother's milk. Animal milk contains a high concentration of minerals and proteins which, if not digest and broken down to smaller particles, they may harm the babies' kidneys.

Once the babies are above a year old, their metabolism can digest most of the nutrients present in milk. The most challenging nutrient is the carbohydrate present in the milk, which is in the form of lactose. And most of the time, lactose stays undigested and gets fermented in the stomach and can make the baby ill. But most of the time, babies excrete the milk from their mouths by vomiting from lactose into lactic. Fermentation is a process where bacteria chemically break down a substance giving out a product. Lactose gets fermented and changed into lactic acid.

Well, the fermentation of milk can be reduced by the help of pasteurization. Most of the time, milk in my country is delivered in a raw form as it is convenient and is available at a mediocre economical rate. Whereas pasteurized milk is sold at a very high price, which cannot be afforded by the commoners. Milk is pasteurized by boiling it first and then cooling it. This will kill many microorganisms and will make milk safe to drink. But in my country, many villagers have problems with being able to afford heat-related appliances.

Well, even though milk is consumed once though it cannot be destroyed. To provide more clarity on this investigation I will survey my nearby village and ask them which type of milk they usually consume, either pasteurized or raw.

Figure1- Pasteurization machine [2]

According to my hypothesis, they might be drinking raw milk due to the unavailability of heat appliances. Raw milk contains an enormous amount of bacteria present in it, which ferments.

[1] https://www.sciencedirect.com/topics/food-science/cow-milk

[2] https://www.sciencedirect.com/science/article/pii/S016041201832381X

RESEARCH QUESTION
How does increasing concentration of anti-biotic affect the rate of fermentation of cow, buffalo and goat milk?
BACKGROUND
Well I was wrong, I got to know by browsing that animal(mammals) other than homo sapiens, like cow, goat and buffalo. Their milk contains high amounts of vitamins (excluding vitamin c), calcium, carbohydrates, minerals and proteins. Which is very healthy and an important source of basic requirement for the growth and the development of the babies and toddlers.
Antibiotics are a range of drugs designed to treat certain infections caused by fighting bacteria in the body. Antibiotic resistance occurs when bacteria alter in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents intended to cure or prevent toxicities. The bacteria survive and continue to divide causing harm and damage. Bacteria can do this through their several mechanisms. Some of them develop the ability to neutralize the antibiotic before it can cause damage, others can swiftly pump the antibiotic out, and still others can alter the antibiotic attack site so it cannot affect the function and province of the bacteria.

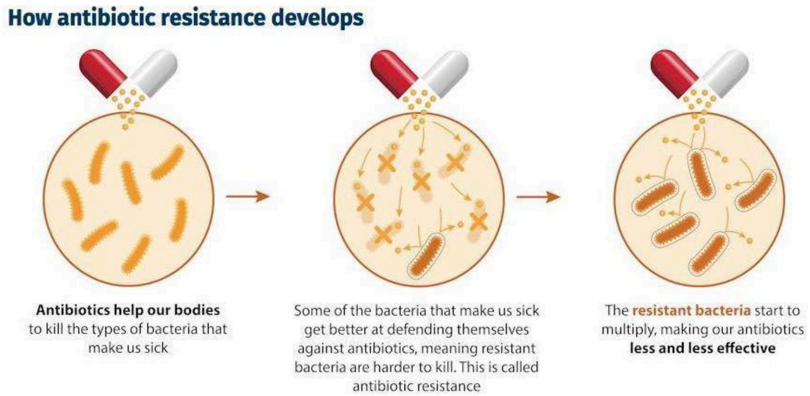


Figure2: -Antibiotic resistance[3]

Antibiotics kill or constrain the growth of susceptible bacteria. sometimes one or two bacteria survive due to their capacity to neutralize or escape the effect of antibiotic; that one bacterium that sustain get multiply and replace all the bacteria by destroying. Subjection to antibiotics therefore provides selective pressure to bacteria, which makes the surviving bacteria more likely to be resistant. In addition, bacteria that were at one-time subject to an antibiotic can acquire resistance through mutation of their genetic material or by acquiring pieces of DNA that code for the resistance properties from other bacteria. The DNA that codes for resistance can be grouped in a single easily transferable package. This means that bacteria can become resistant to many antimicrobial agents because of the transfer of one piece of DNA.[4]
The antibacterial used for the investigation is Amoxicillin oral suspension with the dosage power of 125mg. It is extensively used for remedying infections caused by certain bacteria. It is also used with other medicines to treat ulcers of the small intestines. The low power antibiotic is used for babies.

HYPOTHESIS
Augmentin suspension is a penicillin antibiotic. It works by killing sensitive bacteria. Hence, if mixed with milk, it would definitely resist the bacterial growth of the milk served. The antibiotic added would resist the growth of bacteria in milk to some extent, ultimately slowing down the process of milk fermentation.

Reaction between lactic acid $C_3H_6O_3$ and sodium hydroxide NaOH:

---

[3] https://www.healthnavigator.org.nz/medicines/a/antibiotic-resistance/
[4] https://www.sharecare.com/health/superbugs/how-bacteria-become-drug-resistant

$C_3H_60_3$ + NaOH $NaC_3H_5O_3$ + H_20 ……………………………………..(i)
(Word equation: Lactic Acid + Sodium Hydroxide Sodium Lactate + water)

Hence, the amount of sodium hydroxide used to turn the colour of the phenolphthalein indicator from colourless to pink, would determine the point at with all the lactic acid is neutralized.

VARIABLES

Dependent variable	How was it measured?
Rate of fermentation	It is determined by titrating the milk sample with a strong base Sodium Hydroxide. The more the amount of acid required to reach the state of neutralization, the more lactic acid is produced. Lactic acid is a product of fermentation, hence more lactic acid faster the rate of fermentation.

Independent variable	How was it manipulated?	Unit
Concentration of antibiotic (Augmentin suspension)	This was kept independent as I pre-determined the amount of anti-biotic concentration in the 5 test-tubes. I have used the oral liquid dose which is prescribed by doctor to baby. It ranged from 0ml to 4ml. However, then it was converted the unit to percentage by converting to ratio with the quantity of milk present. Then multiplied the ratio by 100 to ultimately get the percentage of anti-biotic in the sample of milk taken for analysis.	%

Controlled variable	How was it controlled?	Why was it controlled?
Time	The duration of time at which the readings were taken was 24 hours, for 4 days was kept constant. It was done for all the different types of milk.	It was controlled in order to make it possible to compare the rate of fermentation of all the different samples of milk. In finding rate time plays a very significant means of measure.
Milk type	The analysis of only one milk type was done at one point of time. There was no simultaneous work. Basically it was whole different systems of different types of milk. All the different samples of milk were taken fresh in the morning of the day of investigation. The milk was sterilized by boiling it at 100ºC.	The milk types were controlled in order to make is a fair investigation in finding the milk's resistance against the growth of bacteria.
Antibiotic	The antibiotic that was used to vary the concentration is kept constant for all the three types of milk. Augmentin is the antibiotic used.	The antibiotic used for all the milk analysis was controlled in order to give the milk exact same type of immune to resist fermentation. Moreover, it makes the investigation more reliable.
Titrant	Here a strong base NaOH (sodium hydroxide) served as a titrant for all the titrations done.	This was controlled to directly relate the rate of fermentation by the amount of base utilized, in all the cases. It makes the experiment a just one.

Temperature	The entire set up was done under lab conditions for all the different types of milk. The windows were kept closed and the fans were turned off during the entire investigation in order to avoid major changes in temperature.	Bacteria are all very sensitive to even the slightest change in temperature, hence it is mandatory to make sure the temperature for all the setup is same to avoid inappropriate growth of bacteria. Room temperature is suitable to promote bacteria.

MATERIALS REQUIRED

Required Apparatus:

- Rubber cork
- Conical flask
- Burette stand (50 ml uncertainty ±0.05ml)
- Measuring cylinder (500 ml uncertainty ±0.05ml)
- Burette (50 ml uncertainty ±0.05ml)
- Pipette (10 ml uncertainty ±0.05ml)
- Test tubes

Chemicals required-

- 0.1M NaOH (Sodium Hydroxide)
- Phenolphthalein indicator
- Distilled water

Preparation of setup –

- Take a burette stand and attach a burette of 50ml
- The burette was rinsed with NAOH
- Pour NAOH in the burette till the mark of 0 cm3
- The tap of the burette was kept open so that the bubble in the burette is filled
- The burette was refilled till the mark of 0 cm3

This was done to reduce the systematic error that could be present in the experiment.

METHODOLOGY

- Take a 300 ml of raw buffalo milk.
- Take 5 large test tubes and label them 1 to 5.
- Add the antibiotic starting from 0 ml in the first test tube and then 1 ml in number 2, 2 ml in number 3, 3 ml in number 4 and 4 ml in the 5th one.
- Then pipette out accurately 40ml, 39ml, 38ml, 37ml and 36ml of the milk sample and add it to the test tubes numbered 1, 2, 3, 4 and 5 respectively.
- Pipette out 10ml of the milk sample from the beaker and titrate it against 0.1 M NaOH, using the method given below, in order to find the initial acidity of the milk by determining the lactic acid content.
- Cover the test tubes containing milk with different anti biotic concentration with rubber cork.
- Leave the system for 24 hours letting the milk ferment.
- After this time duration, shake the test tubes well.
- Pipette out 10 ml from each of the milk sample of the buffalo milk in the conical flasks.
- Dilute the sample of milk with 10 ml (as directed below)
- After dilution titrate the samples to measure the amount of lactic acid produced.
- Titrate 10 ml of milk samples with varied anti-biotic concentration for 4 days consequently with 24 hours' gap.
- Repeat the entire procedure with the samples of cow and goat milk.

Procedure for the Determination of the amount of Lactic acid in milk:

- Pipette out 10 ml of milk from the test tube number 1 from the raw milk section and pour it in the conical flask.
- Measure accurately 10 ml of distilled water and add it to the milk in the conical flask.
- Then add 2-3 drops of phenolphthalein indicator to the flask.
- Rinse the burette with titrant, NaOH of concentration 0.1 mol/dm^3
- Titrate the diluted milk against NaOH with constant swirling of the conical flask.
- The color change of milk from white to light pink would determine the end point.
- The color variation should be constant for more than 30 seconds and very evident.

The above procedure is to be then repeated using, each sample milk, taken for analysis to determine the amount of lactic acid.

SAFETY AND PRECAUTION

I. **Safety-**

- It is also important to wash hands with antiseptic hand-wash after pouring the anti-biotic into the test-tubes.
- NaOH is a strong base, hence it is important to quickly wash hands under running water if it spill on the hands.
- Wearing gloves serves as one of the measures of precautions.
- Wear lab coats in order to avoid any spill on the uniform.
- Wearing mask so as to prevent any sort of casualties that may harm our face.

II. **Ecological-**

- NaOH, being a strong base, is required to be diluted with cold water before disposing it off as dilution would reduce its caustic effect.
- The left over milk samples, containing antibiotics, were first diluted with water and then disposed of.

III. **Ethical-**

- Milk for investigation had been taken from the animal by a trained professional. Therefore, no animal was harmed.

DATA COLLECTION

Raw data table

<table>
<tr><th colspan="6">Buffalo milk boiled- Day 1</th></tr>
<tr><td rowspan="6">The initial acidity of all the milk without giving time to ferment is same for all</td><td rowspan="2">Amount of milk + distilled water (±0.05)/ml</td><td colspan="2">Burette reading (±0.05)/ml</td><td rowspan="2">Volume of NaOH used (final-initial) (±0.05)/ml</td><td rowspan="2">Concordant reading (±0.05)/ml</td></tr>
<tr><td>initial</td><td>final</td></tr>
<tr><td rowspan="4">10+10</td><td>0.0</td><td>2.1</td><td>2.1</td><td rowspan="4">1.7</td></tr>
<tr><td>0.0</td><td>2.2</td><td>2.2</td></tr>
<tr><td>0.0</td><td>1.7</td><td>1.7</td></tr>
<tr><td></td><td></td><td></td></tr>
</table>

milk with varied antibiotic concentration.					

Buffalo boiled milk- DAY 2

Test tube no.	Milk: antibiotic	Amount of milk + distilled water (±0.05)/ml	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	No change seen	0.0	6.1
2	39:1	10+10	No change seen	0.0	3.2
3	38:2	10+10	No change seen	0.0	2.6
4	37:3	10+10	No change seen	0.0	2.3
5	36:4	10+10	No change seen	0.0	2.0

Buffalo boiled milk- DAY 3

Test tube no.	Milk: antibiotic	Amount of milk + distilled water (±0.05)/ml	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	Slight coagulation	0.0	11.2
2	39:1	10+10	Slight coagulation	0.0	10.8
3	38:2	10+10	No change seen	0.0	3.8
4	37:3	10+10	No change seen	0.0	3.4
5	36:4	10+10	No change seen	0.0	3.2

Buffalo boiled milk- DAY 4

Test tube no.	Milk: antibiotic	Amount of milk + distilled water (±0.05)/ml	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	Coagulated milk	0.0	15.6
2	39:1	10+10	Milk appear clumsy	0.0	7.5
3	38:2	10+10	Foam formation , no coagulation seen	0.0	6.0
4	37:3	10+10	Foam formation , no coagulation seen	0.0	6.6
5	36:4	10+10	Foam formation , no coagulation seen	0.0	6.3

Goat boiled milk- DAY 2					
Test tube no.	Milk: antibiotic	Amount of milk + distilled water	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	Slight coagulation	0.0	8.2
2	39:1	10+10	Slight coagulation	0.0	6.0
3	38:2	10+10	No change seen	0.0	3.2
4	37:3	10+10	No change seen	0.0	3.5
5	36:4	10+10	No change seen	0.0	3.0

Goat boiled milk- DAY 3					
Test tube no.	Milk: antibiotic	Amount of milk + distilled water	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	No change seen	0.0	4.5
2	39:1	10+10	No change seen	0.0	3.8
3	38:2	10+10	No change seen	0.0	3.5
4	37:3	10+10	No change seen	0.0	3.0
5	36:4	10+10	No change seen	0.0	2.7

Goat milk boiled- Day 1					
The initial acidity of all the milk without giving time to ferment is same for all milk with varied antibiotic concentration.	Amount of milk + distilled water (±0.1)/ml	Burette reading (±0.1)/ml		Volume of NaOH used (final-initial) (±0.1)/ml	Concordant reading (±0.1)/ml
		initial	final		
	10+10	0.0	2.0	2.0	2.2
		0.0	2.1	2.1	
		0.0	2.2	2.2	

Goat boiled milk- DAY 4					
Test tube no.	Milk: antibiotic	Amount of milk + distilled water	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	Milk coagulates	0.0	9.4
2	39:1	10+10	Milk appears clumsy	0.0	5.0

Cow milk boiled- Day 1					
The initial acidity of all the milk without giving time to ferment is same for all milk with varied antibiotic concentration	Amount of milk + distilled water (±0.1)/ml	Burette reading (±0.1)/ml		Volume of NaOH used (final-initial) (±0.1)/ml	Concordant reading (±0.1)/ml
		Initial	Final		
	10+10	0.0	2.1	2.1	2.2
		0.0	2.2	2.2	
		0.0	2.2	2.2	

3	38:2	10+10	Milk gets thicker with clumsiness	0.0	4.8
4	37:3	10+10	Formation of foam no coagulation seen	0.0	4.5
5	36:4	10+10	Foam formed , but no coagulation	0.0	4.0

Cow boiled milk- DAY 2					
Test tube no.	Milk: antibiotic	Amount of milk + distilled water	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	Slight coagulation	0.0	10.7
2	39:1	10+10	No change seen	0.0	4.0
3	38:2	10+10	No change seen	0.0	3.9
4	37:3	10+10	No change seen	0.0	3.7
5	36:4	10+10	No change seen	0.0	3.5

Cow boiled milk- DAY 3					
Test tube no.	Milk: antibiotic	Amount of milk + distilled water	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	No change seen	0.0	5.3
2	39:1	10+10	No change seen	0.0	5.0
3	38:2	10+10	No change seen	0.0	2.8
4	37:3	10+10	No change seen	0.0	2.3
5	36:4	10+10	No change seen	0.0	1.8

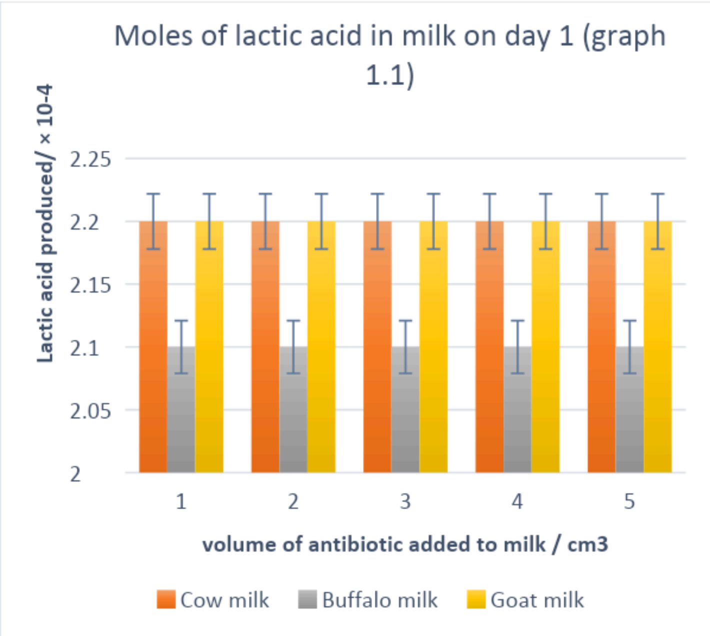
Cow boiled milk- DAY 4					
Test tube no.	Milk: antibiotic	Amount of milk + distilled water	Observation (any qualitative data)	Volume of NaOH used in the burette used for neutralization.	
				Initial	Final
1	40:0	10+10	Milk coagulates	0.0	13.1
2	39:1	10+10	Milk appear clumsy	0.0	11.4

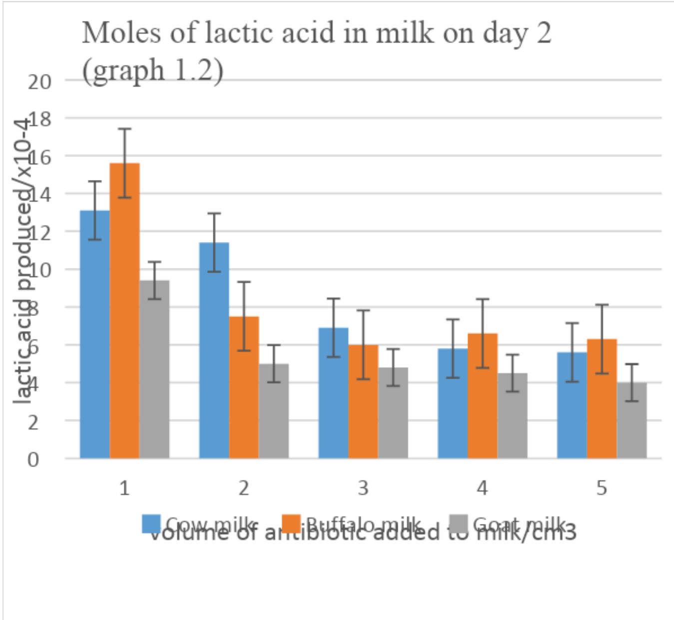
3	38:2	10+10	Milk get thicker	0.0	6.9
4	37:3	10+10	Formation of foam, no coagulation seen	0.0	5.8
5	36:4	10+10	Formation of foam , but no coagulation	0.0	5.6

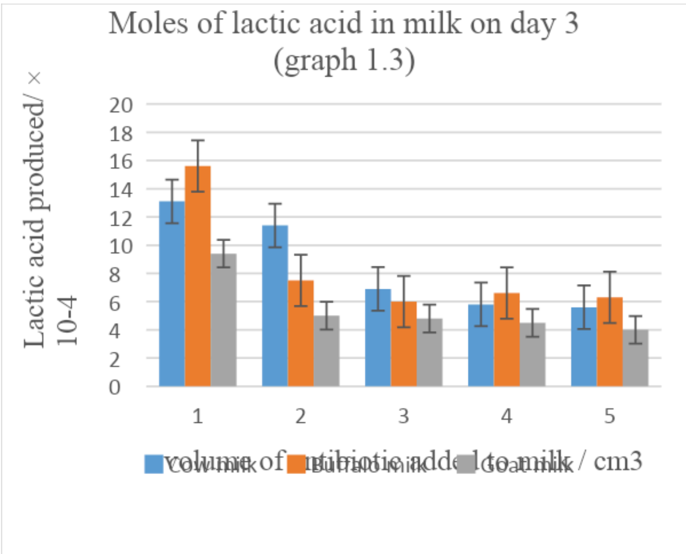
Day 1			
Amount of Anti-bacteria added/ cm^3	Buffalo milk	Cow milk	Goat milk
	Moles of lactic acid produced/mol		
0.00	1.7×10^{-4}	2.2 x 10-4	2.2×10^{-4}
1.00	1.7×10^{-4}	2.2×10^{-4}	2.2×10^{-4}
2.00	1.7×10^{-4}	2.2×10^{-4}	2.2×10^{-4}
3.00	1.7×10^{-4}	2.2×10^{-4}	2.2×10^{-4}
4.00	1.7×10^{-4}	2.2×10^{-4}	2.2×10^{-4}
Day 2			
Amount of Anti-bacteria added/ cm^3	Buffalo milk	Cow milk	Goat milk
	Moles of lactic acid produced/mol		
0.00	6.1 x 10-4	5.3×10^{-4}	4.5×10^{-4}
1.00	3.2×10^{-4}	5.0×10^{-4}	3.8×10^{-4}
2.00	2.6×10^{-4}	2.8×10^{-4}	3.5×10^{-4}
3.00	2.3×10^{-4}	2.3×10^{-4}	3.0×10^{-4}
4.00	2.0×10^{-4}	1.8×10^{-4}	2.7×10^{-4}
Day 3			
Amount of Anti-bacteria added/ cm^3	Buffalo milk	Cow milk	Goat milk
	Moles of lactic acid produced/mol		
0.00	11.2×10^{-4}	10.7×10^{-4}	8.2×10^{-4}
1.00	10.8×10^{-4}	4.0×10^{-4}	6.0×10^{-4}
2.00	3.8×10^{-4}	3.9×10^{-4}	3.2×10^{-4}
3.00	3.4×10^{-4}	3.7×10^{-4}	3.5×10^{-4}
4.00	3.2×10^{-4}	3.5×10^{-4}	3.0×10^{-4}
Day 4			
Amount of Anti-bacteria added/ cm^3	Buffalo milk	Cow milk	Goat milk
	Moles of lactic acid produced/mol		
0.00	15.6×10^{-4}	13.1×10^{-4}	9.4×10^{-4}
1.00	7.5×10^{-4}	11.4×10^{-4}	5.0×10^{-4}
2.00	6.0×10^{-4}	6.9×10^{-4}	4.8×10^{-4}
3.00	6.6×10^{-4}	5.8×10^{-4}	4.5×10^{-4}
4.00	6.3×10^{-4}	5.6×10^{-4}	4.0×10^{-4}

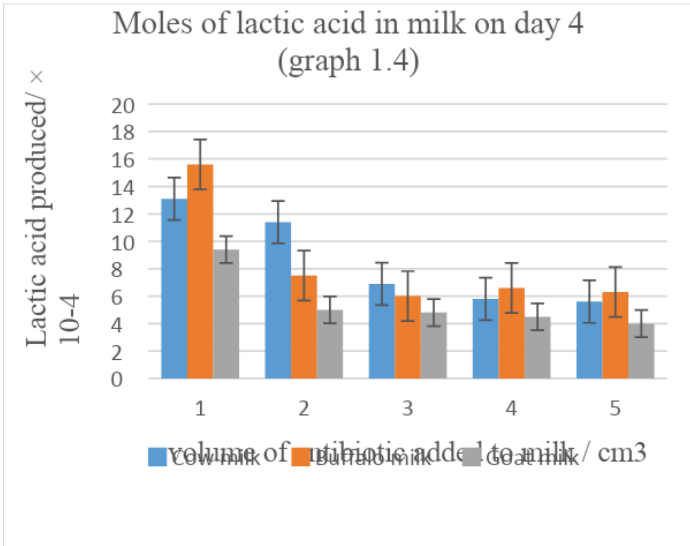
Calculation:
Molar ratio of NaOH and $C_3H_60_3$ is 1:1 according to (balanced) equation (i)
Therefore, the moles of NaOH used would be the same as the moles of lactic acid neutralized.
Formula to calculate moles of NaOH:
n=Cv; C= concentration of NaOH and v= volume in dm^3
Concentration of NaOH: 0.1 mol/dm^3
Sample calculation:
Volume of NaOH= 2.1×10^{-4} dm^3
[NaOH]= 0.1 mol/dm^3
Therefore, moles of NaOH as well as $C_3H_60_3$,
n= 2.1×10^{-3} dm^3 x 0.1 mol/dm^3
n=2.1×10^{-1}

Graphs

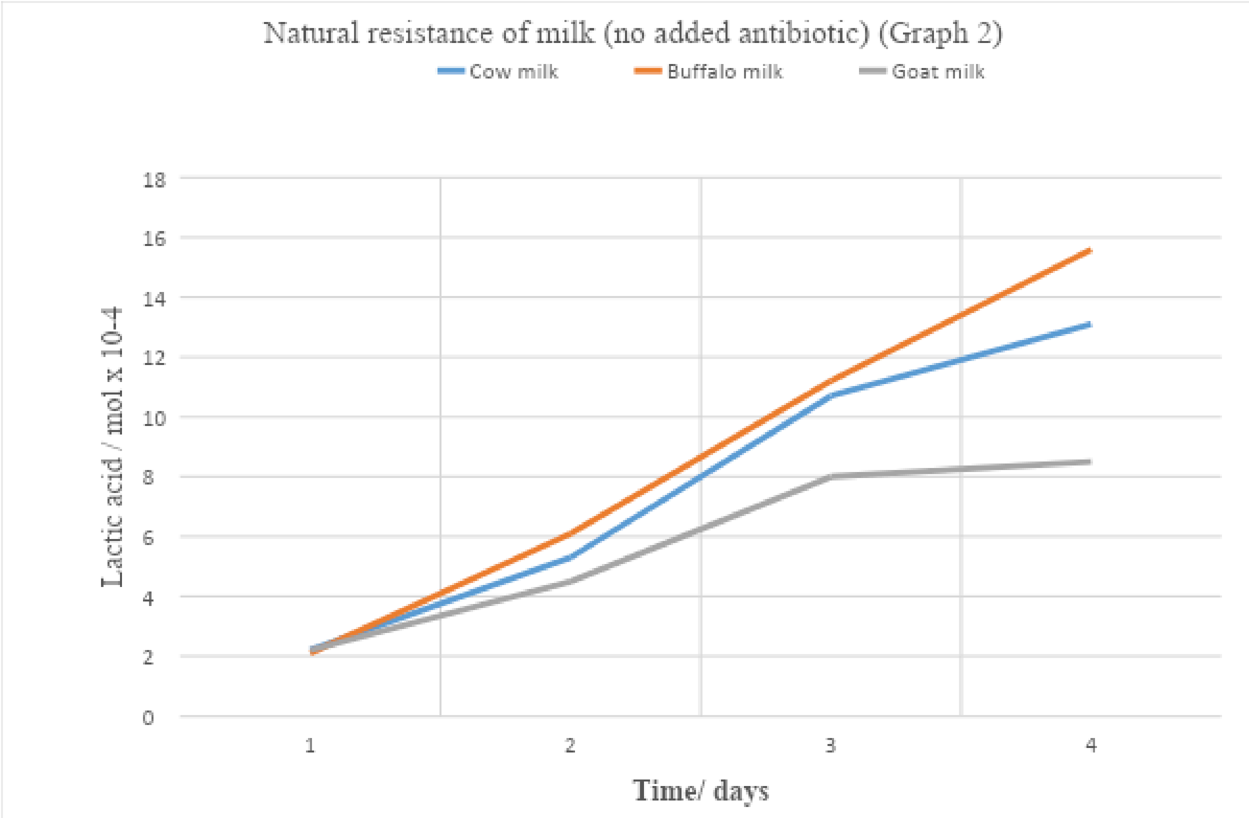








Data indicating the lactic acid production against the volume of antibiotic added to milk for the duration of 4 days.



Data indicating the milk resistance without the addition of antibiotic

ANALYSIS AND CONCLUSION

For the experiment as mention about, I have examined the amount of lactic acid in 3 types of milk, resulted by bacteria activity in the sample of milk. It is possible to make out which sample contained a large amount of lactic acid, by observing the coagulation formed. In this case it was more in buffalo milk as it even used up more amount of NaOH in to acquire the neutralization stage. This also indicates that the bacteria growth has been promoted resulting in the formation of lactic acid.

With the support of some research paper it conclude that[5],the milk which fermented the least and slowest was the goat. However, in the graph (1.1) day 1 it is observed that buffalo have the lowest NaOH content as it 2.1 cm^3 of the base to change the colour of phenolphthalein from colourless to pink. The goat and cow milk had same acidity.

On the second day, clearly observed through graph that there is drastic change in the amount of sodium hydroxide consumed by buffalo milk. After that comes cow milk which shows a gradual increase in acidity. Whereas, goat and cow's milk are on somewhat similar elevation

It is interesting to note that the rate of fermentation of all samples of cow milk is increasing rapidly and reaching up the level of the buffalo milk as witnessed in graph 1.3 and 1.4. Whereas, all the samples with different antibiotic concentration in the milk samples slow their rate of lactic acid in test tube with no antibiotics and the rest. But there is steep fall in acid content in the milk having antibiotic. On the 4th day as witnessed in graph 1.4 all the samples of goat milk still appear to be less acidified compared to other samples.

Moreover, it is also clearly seen that the antibiotic concentration does the affect the rate of fermentation. Though the milk with highest concentration of antibiotic does ferment [6]and

[5] https://www.researchgate.net/publication/248445358_Composition_of_goat_and_sheep_milk_products_An_update

[6] https://www.sciencedirect.com/topics/food-science/milk-from-animals

become acidic, however the rate is comparatively very slow with increasing antibiotics. This further makes the hypothesis which states that, “the antibiotic would slow down the rate of fermentation of milk “holds the statement truly.
From the above analysis, it can be inferred that goat milk is suggestive to be having a natural resistance against the bacterial growth. This is evident by complementary graph 2 which shows the natural fermentation rate with no added antibiotic intervention.
The significance of this research is to perpetuate that what happens when an antibiotic is taken through varied types of milk. All in all, it showed that with the antibiotic concentration rate of formation slows down

<u>LIMITATION</u>
To make this investigation more reliable and cogent if repeated, one should consider the fact that the current investigation has got several limitations and how these could be improvised.

Limitations (error)	**Sources of error**	**Scope of improvement and error reduction**
Systematic error	Faulty apparatus such as poorly calibrated burette and measuring cylinder	Proper shortage of apparatus without the zero error along with regular maintenance.
Random error	Errors during titration	In order to lessen such random error it is mandatory to repeat the titration of the milk sample for at least 3 times and then get a concordant constant reading
Random error	Only three types of milk underwent analysis	Instead of limiting the milk type to cow, buffalo and goat, one could inculcate other milk as well such as that of camel, sheep etc. Doing so would contribute in establishing a trend in the bacterial resistance of milk.
Random error	The chosen samples are not representatives as only milk was taken from one animal from whole of their species and one locality	The measurements should be repeated within the same locality and also from different localities.
Systematic error	Parallax error in reading the Burette	This can be reduced when the burette is read from the meniscus from a 180 degree angle.
Random error	Amount of indicator varied	Indicator being a buffer solution if poured more can give incorrect reading of titration. It is important to carefully only use 2 drops for the colour change.
Systematic error	Using a titrant to determine acidity of milk samples	A more refined means of measurement such as a pH sensor can be used instead.

<u>FURTHER SCOPE OF INVESTIGATION</u>
This investigation can further be made more reliable with scope of critical analysis by repeating it with using the raw milk. One can use the milk without sterilizing it and then comparing the bacterial resistance of both the boiled and the raw milk. The investigation can be kept same by using the same variables but the change in their presentation for the setup as-

1- <u>Rate of fermentation</u>- Keep this constant because the state of neutralization will only be visible when loaded with strong base.
2- <u>Concentration of antibiotic (Augmentin suspension)-</u> This can be changed as the data collected from this experiment make conclusion only for the antibiotic based on babies. So instead of Augmentin there can be tablets used or any prescribed antibiotic as Doxycycline which is taken by adults when suffered from upset stomach.

But the controlled variable can also be manipulated as the milk type can be change any other milk according to the accessibility. The temperature and time factor can be taken into more environmental rather than a lab set-up to provide more insight into the result.

BIBLIOGRAPHY

1, https://www.researchgate.net/publication/248445358_Composition_of_goat_and_sheep_milk_products_An_update. Accessed 20 Oct 2019.

2"Milk from Animals - An Overview | Science direct Topics". *Sciencedirect.Com*, 2020, https://www.sciencedirect.com/topics/food-science/milk-from-animals. Accessed 19 Oct 2019.

3"How Do Bacteria Become Resistant To Antibiotics? | Superbugs". *Share care*, 2020, https://www.sharecare.com/health/superbugs/how-bacteria-become-drug-resistant. Accessed 20 Oct 2019.

4- https://www.researchgate.net/profile/Ginevra_Gravili. Accessed 20 Oct 2019.

5- Course Hero". *Coursehero.Com*, 2020, https://www.coursehero.com/home/welcome/. Accessed 20 Oct 2019.

6-"Understanding Antibiotic Resistance". *Open learn*, 2020, https://www.open.edu/openlearn/ocw/mod/oucontent/view.php?id=75461. Accessed 20 Oct 2019.

7-https://www.healthnavigator.org.nz/medicines/a/antibiotic-resistance. Accessed 21 Oct 2019.

8- https://www.sciencedirect.com/science/article/pii/S01604120183238. Accessed 21 Oct 2019.