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Study of some Indigenous and Exotic Cattle Breeds and a Comparative Analysis of their Milk Quality using MBRT

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Abstract: ***Dairy breeds or milch breeds are high milk yielding cows and we selected five breeds such as Holstein-Friesian (HF), Jersey, Red Dane, Sahiwal, Swiss Brown and two cross breeds such as JerseyXHF and SunandiniXHF from Aiswarya Dairy farm, Anchal for qualitative analysis of milk samples. Among these Holstein-Friesian (HF), Jersey and Swiss Brown are popular exotic breeds reared in India. Milk can be graded by physical, chemical and microscopical examinations. The methodology employed the enzymatic reduction of methylene blue by a metabolically active organism turning the Methylene Blue colorless. The principle of methylene blue reduction test depends on the fact that the color imparted to the milk by adding a dye such as methylene blue will disappear more or less quickly, which depends on the quality of the milk sample to be examined. Methylene blue is a redox indicator, that lose its color under the absence of oxygen and is thought to be reduced. The depletion of oxygen in the milk is due to the production of reducing substances in the milk due to the enhanced rate of bacterial metabolism. The dye reduction time refers to the microbial load in the milk and the total metabolic reactions of the microorganism.***

Keywords: Milch breeds, MBRT, HF, Decolourisation, Incubation time

I. INTRODUCTION

In India, the rural population depends heavily on the livestock for their livelihood. Among the livestock, dairy cattle play a pivotal role in the economy of rural poor (Nair, 1973). Dairy farming provides a livelihood for a large section of people across our country. In the selection of suitable dairy animals criteria such as breed, pedigree, production records and physical appearance are to be considered (Kunzi, 1984., May et al., 2003; Menzy et al., 1982). Apart from a large number of non-descript breeds, there are 26 well defined breeds of cattle and 6 breeds of buffaloes in our country (Patel, 1976; Sinha., 1951). Some of the important breeds are Sindhi, Sahiwal, Gir, Jaffarabadi, Jersey, HF, Sunandini, Swiss brown, Red Dane etc. Milk is a good medium for the growth of microorganisms. A variety of microorganism can be found in both raw milk and pasteurized milk. These actively growing microorganisms reduce the oxidation reduction potential of the milk medium due to the exhausted oxygen by the microorganism. Normally the milk is contaminated with microorganisms such as Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeroginosa, Enterobacter spp., Bacillus spp., Paenibacillus spp., etc. Contaminated milk is one of the important sources for transmission of diseases from animals to humans. The main reason for this contamination is the un-proper handling of milk. Normally milk is contaminated during the milking process by the microorganisms present in the exterior surface of the animals, pipelines such as udder and adjacent areas. Unsterilized dairy utensils such as milking machines, milk cans are also a good source of contamination by the microorganism (Cappuccino and Natalie, 2002). The milk contains energy sources such as lactose (sugar), nitrogenous compounds such as proteins, amino acids, ammonia, urea etc. for the growth of microorganism. Acid fermentation by the bacteria is common under ordinary conditions. Souring of milk indicates the milk is spoiled. Acid formation in the milk is indicated by the sour flavor, coagulation of milk to give a jelly like curd appearance or clear whey nature. Lactic acid fermentation is common in the raw milk at the room temperature. At temperature from 10 to 37 ^{o}C souring is mainly due to Streptococcus lactis, Enterococci, Lactobacilli and other coliform bacteria. At temperatures from 37 to 50 ^{o} C the most common contaminants of milk are S. faecalis and S. thermophilus. Thermophilic bacteria such as L. thermophilus can grow in the milk at higher temperatures (Nandy and Venkatesh, 2010). Pasteurization is an important process to kill most acid producing microorganisms while permitting the growth of heat resistant microorganisms such as Streptococcus thermophilus and Lactobacilli. Other acid producing microorganisms are Micrococcus species, Bacillus species (mainly lactic acid) and Clostridium species (mainly Butyric acid) (Chacko and Jose, 1988). Microorganisms such as Clostridium spp. and Bacillus spp. can also produce gases such as hydrogen and carbon dioxide which can be indicated by the formation of foaming at the top of the milk suspension. The other way of milk contamination is the hydrolysis of milk proteins by the growing microorganisms. The release of peptides in the milk leads to a bitter

flavor to the milk. Ropiness, sliminess in the milk, is caused by the release of slimy capsular material from the cells. Enterobacter aerogenes, E. coli, Micrococcus freudenreichii are examples of microorganisms that can cause ropiness in the milk. Oxidation of unsaturated fatty acids can also lead to change in odor and taste of milk. Production of alkaline products such as ammonia, urea, carbonates etc can also produce off flavors to milk (William et al., 1998). Milk can be graded by physical, chemical and microscopical examinations. The Methylene blue reduction test (MBRT) is a chemical test for counting the germs in milk. It is based on the fact that the coloring matter, methylene blue is blue in the presence of oxygen. When oxygen is removed from milk to which the dye is added, the dye immediately loses its colour (Chacko et al., 1988, 1992). The bacteria that are ordinarily found in milk, use oxygen in their growth and multiplication. Many germs will quickly use up all the oxygen, while a small number will require a much greater length of time. Fresh milk has a considerable amount of oxygen dissolved in it. If the dye methylene blue was added to fresh milk, it will turns to a blue colour. This blue colour will remain until all the oxygen is used up, then the milk will almost immediately change blue to white again. The larger the number of bacteria in milk, the sooner the colour change takesplace. Hence the use of methylene blue becomes a valuable test for determining the relative number of bacteria present in a given number of milk samples. The formation of Methylene blue reductase is thus becoming a popular tool for determining the quality of the milk (Hatch, 1927).

A. During incubation, observe colour changes as follows:

*1)*If any sample is decolourized on incubation for 30 minutes, record the reduction time as MBRT - 30 minutes.

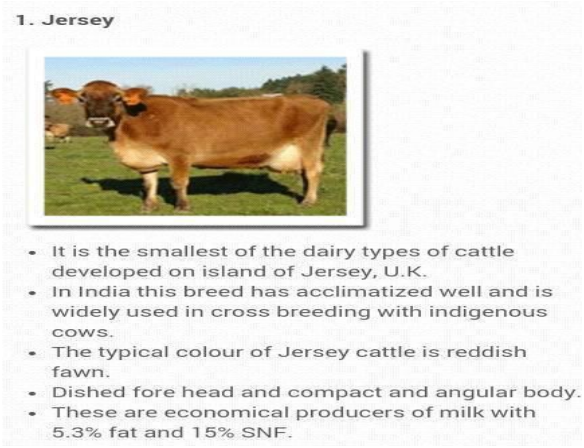
*2)*Record such readings as, reduction times in whole hours. For example, if the colour disappears between 0.5 and 1.5 hour readings, record the result as MBRT - 1 hour; similarly, if between l.5 and 2.5 hours as MBRT - 2 hour and so o

*3)*c) Immediately after each, reading, remove and record all the decolourized samples and then gently invert the remaining tubes if the decolourization has not yet begun. Disappearance of color with limited time indicates the absence of oxygen in milk. Refrigerated milk contains more oxygen than warm milk. At the time of milking process, it has more oxygen content than the other cases. Rate of reduction depends on the nature of the organism present in the milk. The rate of reduction by different microorganism is arranged in order below.

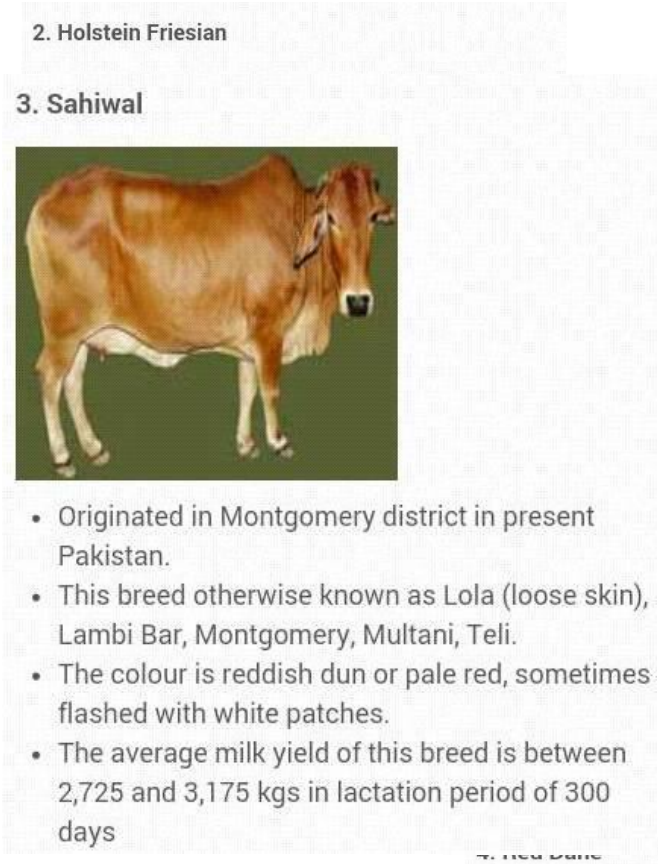
B. Coli (coliforms) > streptococcus lactis > faecal streptococci > micrococci > thermoduric organisms > psychrotrophic organisms

Aiswarya dairy farm is situated in Thumbodu near Bharathipuram, Yeroor. The farm gives all the facilities for the neat and hygienic maintenance of dairy animals. The farm consists of 20 cows. Most of them are indigenous and some exotic breeds are also there. The indigenous breeds include Gir (Gujarat), Sahiwal (Hyderabad), Vechur (Kerala) and Kasaragod Kullan (Kerala). Among these the milk of Vechur has high medicinal quality and economic value. The exotic breeds found in that farm include Jersey, Holstein Friesian, Brown Swiss, Red Dane etc. All these breeds have their own identification features. Cross breeds like Sunandini X HF and Jersey X HF were also found. Sunandini is a composite breed of cattle developed in India by crossing non descript cattle with exotic breeds like Brown Swiss, Jersey, HF etc. We collected milk samples for our experiments from pure breeds such as HF, Jersey, Red Dane, Brown Swiss and Sahiwal. Among these HF, Jersey, Red Dane and Brown Swiss were exotic breeds and Sahiwal is an indigenous breed. The crossbreed samples we collected include Jersey X HF and Sunandini X HF.

II. INDIGENOUS AND EXOTIC BREEDS OF CATTLE



(FIG1 and 2. SAHIWAL and JERSEY : Adapted from "Breeds of Livestock, Department of Animal Science" www.ansi.okstatae.edu, 2017)



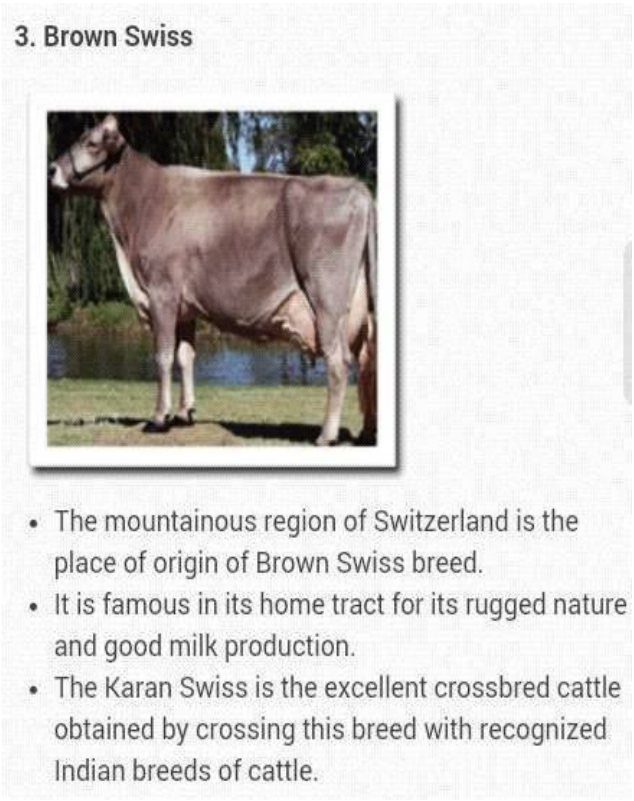


Fig 3, 4 and 5. Holstein friesian, brown swiss and red dane: adapted from "breeds of livestock, department of animal science" www.ansi.okstatae.edu, 2017)

III.OBJECTIVE

To compare the breed wise quality of milk samples from indigenous and exotic breeds of cattle.

A.Materials Required:

Milk samples to be analyzed, Methylene blue reductase test (MBRT) dye solution (dye concentration 0.005%), Test tubes, Test tube rack, Measuring cylinders (10 ml), Dropper, Water bath (42±1^{0}C), Cotton, Bunsen burner

B.Procedure

The test has to be done under sterile conditions. Take 10 ml milk sample in sterile MBRT test tube. Add 1 drop of redox indicator MBRT dye solution (dye concentration 0.005%) to each test tube containing milk sample. Tighten the test tube mouth with cotton swab. Gently shake the tubes at about four or five times to ensure proper mixing of the methylene blue solution. Keep the tubes in the water bath at 42±1^{0}C. This time is recorded as the beginning of the incubation period. Note the incubation time. Incubation time is the time elapsed for the colour to turn whitish appearance (Decolourization is considered complete when only a faint blue ring (about 5mm) persists at the top. Stabilize the tubes for 5 minutes.

MBRT Procedure

IV. RESULTS OBTAINED

(1)Comparison of milk quality between indigenous, exotic and cross breeds (Without Refrigeration)

Name of breeds		Total incubation time	Quality
INDIGENOUS	Sahiwal	40 Minutes	POOR
EXOTIC	HF	40 Minutes	POOR
	Jersey	40 Minutes	POOR
	Red Dane	40 Minutes	POOR
	Swiss Brown	1 Hour 30 Minutes	FAIR
CROSS	Jersey X HF	1 Hour 5 Minutes	FAIR
	Sunandini X HF	50 Minutes	POOR

(2)Comparison of milk quality between indigenous, exotic and cross breeds (After refrigeration for 1 Hour)

Name of breeds		Total incubation time	Quality
INDIGENOUS	Sahiwal	20 Minutes	VERY POOR
EXOTIC	HF	50 Minutes	POOR
	Jersey	40 Minutes	POOR
	Red Dane	30 Minutes	POOR
	Swiss Brown	2 Hours 15 Minutes	GOOD
CROSS	Jersey X HF	55 Minutes	POOR
	Sunandini X HF	40 Minutes	POOR

V.DISCUSSION

Methylene Blue Dye Reduction Test, commonly known as MBRT test is used as a quick method to assess the microbiological quality of raw and pasteurized milk. This test is based on the fact that the blue colour of the dye solution added to the milk get decolourized when the oxygen present in the milk get exhausted due to microbial activity. The sooner the decolourization, more inferior is the bacteriological quality of milk assumed to be. This test is widely used at the dairy reception dock, processing units and milk chilling centres where it is followed as acceptance/rejection criteria for the raw and processed milk. Methylene Blue Dye Reduction Test (MBRT) has ata in evaluating cell viability in a very short time (Nandy et al., 2007). In the current study, MBRT was adopted to develop a protocol for determining the quality of milk samples from different breeds of cattle. The modified protocol was extended to demonstrate the qualitative comparison of milk samples from indigenous, exotic and cross breeds. The methodology employed the enzymatic reduction of methylene blue by a metabolically active organism turning the Methylene Blue colorless. The rate of decolouration by the metabolically active cells can be correlated to the number of viable cells. For this purpose, the slope of the MB decolouration rate was calibrated with respect to colony forming units (CFU) obtained through plating. This method was successfully employed to characterize the viability of E. coli and B. subtilis (Bapat et al., 2006). Studies revealed that MBRT can be successfully employed to quantify viable cell count in a very short time (less than 4min). Methylene Blue (MB) dye has been employed to check for the overall microbial load and quality control of milk and other liquid foods (Impert et al., 2002). Because of its size and positive charge, it does not enter into the cells appreciably. It gets reduced to 'leuko' or colourless form of MB at the cell surface via reductase enzymes present in the cell membrane. This colourless form of methylene blue (MBH) is uncharged, lipophilic, and enters cells by diffusion across the plasma membrane where it is re-oxidized and thus sequestered within the cells (May et al., 2003). If oxygen is available, reduced MB can be oxidized by the mitochondrial electron transport system. This will result in the reappearance of the blue color. Up to now, the exact mechanism of dye reduction is not known, but some reports available suggest that MB is reduced by transmembrane reductases (Bongard et al., 1995; Merker et al., 1997). This mechanism is applied to evaluate the microbial load in a liquid medium. The shorter time required for the disappearance of the blue colour is indicative of a higher microbial load. It is assumed that greater the number of microorganisms, more the oxygen demand and lesser the oxygen concentration in the medium resulting in the faster disappearance of the colour. This fact has been used as a broad indicative test of a microbial load representing microbial quality of milk. There are so many factors plays significant role in the reduction of methylene blue in milk. Microbs such as bacteria may play but an insignificant part in the reduction of methylene blue in milk, though their de-oxygenating effect may be of influence in the commercial application of the test (Jepras et al., 1997). Milk as it exists in the udder, or milk drawn anaerobically, reduces methylene blue almost instantaneously, whereas the same milk exposed to oxygen will usually take more than ten hours to reduce. The oxidation-reduction potential of anaerobically drawn milk is much lower than the same milk exposed to oxygen, and in accordance with its atalyze toward methylene blue (Bapat et al., 2006). Evidence is given for the presence of a redox system, present in low concentration, as responsible for the reduction of methylene blue. Although the addition of small amounts of cysteine or glutathione to milk leads to the reduction of methylene blue, their

absence from milk excludes them as possible factors in the normal reduction. The possibility that lactoflavin may furnish the redox system is suggested (Bongard et al., 1995). The reduction of methylene blue in milk is also atalyzed by light in the visible spectrum. The presence of light fastens the reduction rate; hence the test tube under observation should be tightened properly (Cappuccino and Natalie, 2002). Uniform concentration of methylene blue dye should use in all test samples since addition of more methylene blue dye will result in more reduction time. Increased incubation time reduces the reduction time since the activity of some organism increases with increased incubation temperature. The test tubes, periodically invert at regular intervals during incubation time to improve the accuracy of the test result. Otherwise microorganisms may not be evenly distributed in the milk sample leading to wrong result interpretations (William et al., 1998).

VI.SUMMARY AND CONCLUSION

We expected that the milk quality of indigenous breeds were greater than that of exotic breeds. But our results showed that the milk quality of the indigenous breed, Sahiwal was very poor when compared to other exotic and crossbreeds taken. It may be due to some factors such as the contamination of microorganisms, light exposure during milk transferring etc. So from our results we conclude that the MBRT is very valuable and efficient test for determining the quality of milk samples. The advantage of the test is that the test can be easily made by anyone of ordinary skill and intelligence.

VII. ACKNOWLEDGEMENT

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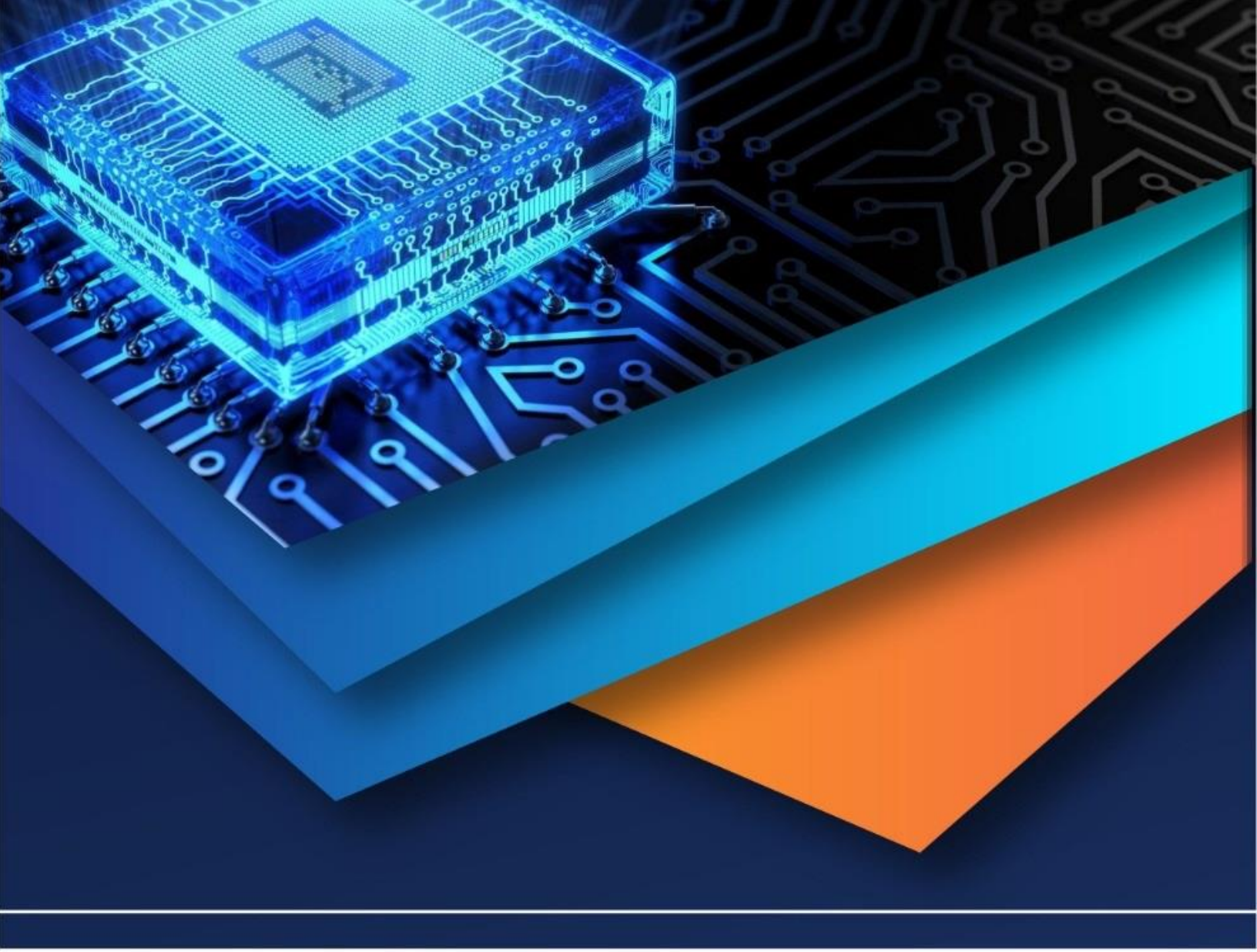

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