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CONCENTRATION OF TRACE ELEMENTS IN RAW MILK OF WILD AND HYBRID COWS IN GANJAM DISTRICT

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ABSTRACT

The milk samples of wild and hybrid cows were collected randomly in different small and marginal farms of Berhampur city of Ganjam district, Odisha. Concentration of magnesium, calcium, potassium, sodium, copper and iron were determined from the milk samples. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used for the analysis of milk samples to detect the concentration of trace elements. 10 milk samples each from wild and hybrid cows were taken separately for the study. Average concentrations of trace elements in raw milk of wild cows were the following: Calcium- 1229 ppm, Magnesium 136.3 ppm, sodium 395.3 ppm, Potassium 1415 ppm, iron 1.51 ppm, and copper 0.67 ppm. Average concentrations of trace elements in raw milk of hybrid cows were the following: Calcium- 1304 ppm, Magnesium 141.7 ppm, sodium 396.9 ppm, Potassium 1427 ppm, iron 1.99 ppm, and copper 0.71 ppm. The concentration of trace elements like calcium, Potassium and Iron are higher in hybrid cows as compared to the wild cows. The result found a significant increase ($P<0.001$) in calcium and iron contents in the milk of hybrid cows in comparison to wild cows. Similarly, a significant increase ($P<0.05$) in magnesium and potassium contents of milk in hybrid cows were observed when compared with wild cows. Whereas a non-significant (P, NS) increase in sodium and copper contents in the milk of hybrid cows were observed in comparison to wild cows.

INTRODUCTION

According to Oxford dictionary the synonym of ‘Hybrid’ and ‘wild’ is ‘Cross breed’ and ‘indigenous’. Hybrid is a plant or animal that has parents of different species or varieties. Crossbreeding is the process of hybridising two different species, breeds or varieties. The offspring produced is known as crossbreed (Wakchaure *et al.,* 2015). The productivity of wild or indigenous breeds can be improved by introducing foreign genes (Gitau, 2014). All the domestic cows on earth are descended from a single species of wild cow called *Bos primigenius*. This wild cow is now called as aurochs or urus. The auroch is ancestral to both zebu and taurine cattle. Indigenous is a plant or animal that originated or occurred naturally in a particular place (NABRC, 2017).

Milk contains different types of trace elements which are highly essential for the growth and development of adults and children. Milk and dairy products are consumed by the human being worldwide. Milk is one of the most essential sources of diet especially for the children and infants. Elements like Cu, Cr, Se and Mn are cofactors for few enzymes and they have very important role on human health. Therefore daily intake of all these elements must follow the values of Recommended Dietary Allowance (RDA) for the maintenance of adequate nutritional level (Saribal, 2019). Trace minerals concentration are very low in a particular sample. The trace minerals play a major role in animal nutrition. Some trace elements like calcium and phosphorous are required in large quantity whereas others like iodine requirement is very low (Nileshkumar and Subha, 2018). In recent years, trace elements have gained importance as biomarkers in many chronic diseases. Unfortunately, the requirement for sample volume increases with the extent of investigation either for diagnosis or elucidating the mechanism of the disease. (Mohammed Nawi *et al.,* 2020). The trace elements have significant role in the unified systems of oxidative metabolism, energy metabolism and immune function in ruminants. The trace minerals like Cu, Mn, Zn and Se has important role as the components of proteins and antioxidant enzymes (Overton and yasui, 2014).

Some toxic elements were found in the cow milk which leads to environmental contamination (Pilacrzyk *et al.,* 2013). These days cow's milk has taken more widely for the study of trace elements, mostly in polluted and industrialized areas. So it was also considered as a very good bio-indicator of contamination for the agricultural atmosphere (Elbagermi *et al.,* 2014; Dobrzański *et al.,* 2005). Trace elements are more dangerous to human being as they are accumulated in the food chain and persevere in the environment. For the analysis of its toxicological aspects, necessary steps must be taken to know its concentration in the diet and their state of oxidation (Hughes *et al.,* 2011). Milk and its products contains very essential nutrients for the human as its consumption has been increased in last few years. All these products have very good quantity of calcium and high biodisponibility. Numerous dangerous compounds or elements like metalloids and metals were collected with the food chain (Birghila *et al.,* 2008).

According to Pérez-Carrera *et al., (*2016), forage and soil's minerals may contribute their occurrence in cow milk. According to RDA, the status of dietetic elements like Cu, Zn, Cr and Se in milk and its products are making good contribution to consumer's diet (Khan *et al.,* 2014). These trace elements are a group of metalloids and metals with the atomic thickness more than 6 g/cm. The term is broadly accepted and applied with the elements just like copper (Cu), cadmium (Cd), lead (Pb), Zn and Fe. These are usually linked with toxicity and create pollution (Malhat *et al.,* 2012). For analysis of milk, ICP-OES technique is used with acid digestion. A lot of hazardous elements like metalloids and metals are accumulated in the food chain. So the concentrations of these pollutants are increased in the surroundings through agriculture, urbanization and industrialization (Birghila *et al.,* 2008). Different concentration of trace elements were observed in many diseases like breast cancer (Adeoti MI *et al.,* 2015), acute leukaemia (Kim *et al.,* 2017), the pathological events is associated with trace elements through genotoxicity activation (Lin and Giovannucci, 2014), diabetes (Cancarini *et al.,* 2017) and Parkinson's disease (Zhao *et al.,* 2013). Milk is a compound of coactive material which enhances the development and growth of infants. It is an excellent source of fat, protein, minerals, carbohydrates and vitamins. Therefore milk is an important component for human diet (Buldini *et al.,* 2002; Qin *et al.,* 2009; Enb *et al.,* 2009). Milk is contaminated by some heavy metals with water through packaging and manufacturing process (Ayar *et al.,* 2009;

Anastasio *et al.,* 2006). Earlier, trace elements levels were analysed in different human biological samples such as hair [Wei *et al.,* 2014], nails (Przybylowicz *et al.,* 2012), tissues (Sohrabi *et al.,* 2018) and cerebrospinal fluid (Sanyal *et al.,*2016), urine (Saravanabhavan *et al.,*2016), saliva (satija *et al.,*2016), blood (Mohammed *et al.,*2017)) for mapping the toxicological profiles (Keil *et al.,*2011) and measuring the professional exposures(Dartey *et al.,*2017).

Milk is the most essential source of food across the World. So its monitoring is essential for the detection of heavy metals as it is important for toxicological, nutritional and environmental purpose. Milk carries a numbers of xenobiotic substances which represent a scientific threat for the wellbeing of human. Investigation of the heavy metal concentrations in the milk is a very good "direct indicator" and also an "indirect indicator" for the pollution level of the environment (Licata *et al.,* 2004; González- Montaña *et al.,* 2012). In most of the studies, the concentration of trace elements and toxic metals were detected in the milk of exotic breed cows. As few trace metals in milk are not suitable for the human body like As, Cd, Se, Pb and Cr (Leotsinidis *et al.,* 2005; Ayar *et al.,* 2009).

Materials and Methods

Study area and collection of samples

The present study was conducted during October 2019 to January 2020. Twenty milk samples (ten wild and hybrid cows each) were collected randomly from different small and marginal farms of Berhampur city, Ganjam, Odisha. The selected breeds of cows were belonged to the similar age group (4-7 years old) and similar lactation period (2^{nd} to 4^{th} lactation). Before the collection of milk, all the bottles were initially cleaned with water and detergent. Again these bottles were cleaned with deionised water and allow them to dry. All the milk samples were collected and put into the sterile bottles and stored at 4 °C until analysis. All the samples were analysed on the same day of collection.

Instrumentation

For the analysis of trace elements like calcium (Ca), Potassium (K), copper (Cu), Magnesium (Mg), Sodium (Na) and Iron (Fe), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used. For analysis, a Thermo Scientific™ iCAP™ 7400 ICP-OES Duo was used together with an aqueous sample introduction kit. A Teledyne CETAC ASX-560 Auto sampler was used to automatically transfer the sample to the ICP-OES.

The instrumental setting and operative condition are given in table 1.

Table 1. Instrument Parameters

Parameter	Setting	
Pump Tubing (Standard Pump)	Sample Tygon® orange/whiteDrain Tygon® white/white	
Analysis Pump Speed	50 rpm	

Spray Chamber	Glass Cyclonic	
Nebulizer	Glass Concentric	
Nebulizer Gas Flow	0.5 L min-1	
Coolant Gas Flow	12 L min-1	
Auxiliary Gas Flow	0.5 L min-1	
Centre Tube	2 mm	
RF Power	1150 W	
Plasma View	Axial	Radial
Exposure Time	UV 15 s, Vis 5 s	Vis 5 s

The instrument was allowed 15 minutes for warm up, after the ignition of the plasma. Directly spectrometer optimization was performed prior to the analysis and the samples were analyzed. ICP-OES was used to determine all the periodic table elements. It has an extensive energetic concentration range to detect elements from low to high concentration. All samples were running once without further dilution. The Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software was used to perform the analysis and evaluate the data quantitatively. It has matrix tolerance and enhanced the speed of analysis that can be achieved (Sanja Asendorf, 2017).

Preparation and digestion of samples :

Acid digestion with HNO_3 and H_2O_2 (EPA 3050)

To detect trace element's concentrations, a hot block method was used. The EPA 3050 method was proposed by USEPA (1998). For dilution, mineralization and digestion of the milk, HNO_3 and H_2O_2 was used. Deionised water was used for sample preparation. The elements (iron, calcium, copper, potassium, sodium and magnesium) were analysed by ICP-OES. To remove the organic portion of milk, 0.5ml of cow milk has treated with 5 ml of 65% HNO_3 and 3 ml 30% H_2O_2 and digested in Microwave Digestion System. The temperature of the mixture was gradually increased from 90 degree to 120 degree Celsius till the brown fume appeared which indicates complete oxidation of organic matter. After cooling, the mixture was filtered with Whatman filter paper No. 42. Then the mixture was transferred to polypropylene tubes. Each filtered samples were diluted to 10ml with doubled deionised water. Then the samples were analysed by ICP-OES.

Statistical Analysis

The data was statistically analysed with Microsoft excel (MS Office 2007). The concentrations of trace elements were compared by the student's t-test. All the data were calculated as an arithmetic mean, minimum value, maximum value, STDV and SEM.

Result:

Table 2: Content of trace elements (calcium, magnesium sodium, potassium, iron, and copper) in raw milk of wild and hybrid cows. Value for Mg, Ca, K, Na, Cu and Fe are in ppm (Mean ± SEM). Numbers in parentheses indicate sample size, NS, not significant at 0.05 confidence level.

Category	Ca	Mg	Na	K	Fe	Cu
Wild cow	1229 ± 8.94	136.3 ± 1.46	395.3 ± 2.82	1415 ± 3.2	1.51 ± 0.04	0.67 ± 0.01
	(10)	(10)	(10)	(10)	(10)	(10)
P	P < 0.001	P < 0.05	P , NS	P < 0.05	P < 0.001	P , NS
Hybrid cow	1304 ± 5.013	141.7 ± 1.776	396.9 ± 2.523	1427 ± 4.069	1 .998 ± 0.042	0.711 ± 0.018
	(10)	(10)	(10)	(10)	(10)	(10)

Figure -1: Concentration of calcium in raw milk of wild and hybrid cows. Value of calcium concentration is in ppm. Columns represent the mean values and vertical bars SEM.

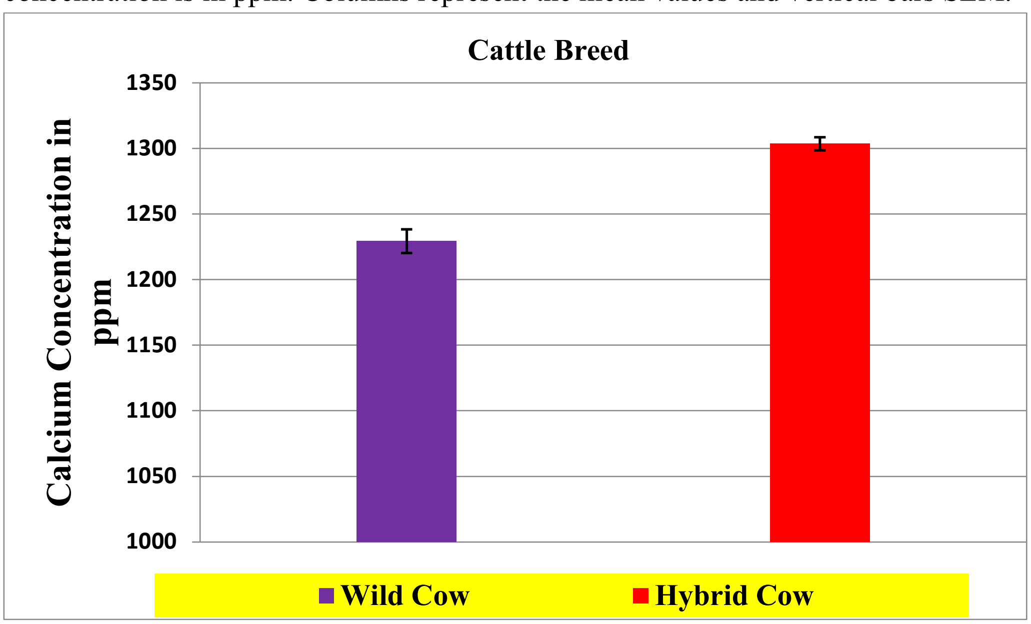


Figure -2: concentration of magnesium in raw milk of wild and hybrid cows. Value of magnesium concentration is in ppm. Columns represent the mean values and vertical bars SEM.

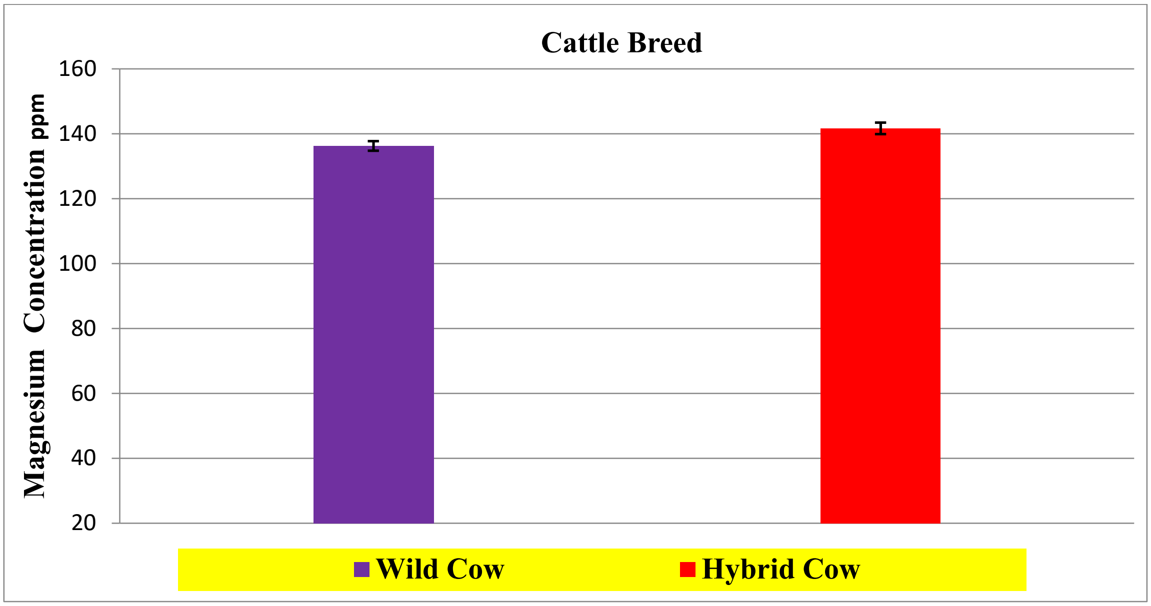


Figure -3: Concentration of sodium in raw milk of wild and hybrid cows. Value of sodium concentration is in ppm. Columns represent the mean values and vertical bars SEM.

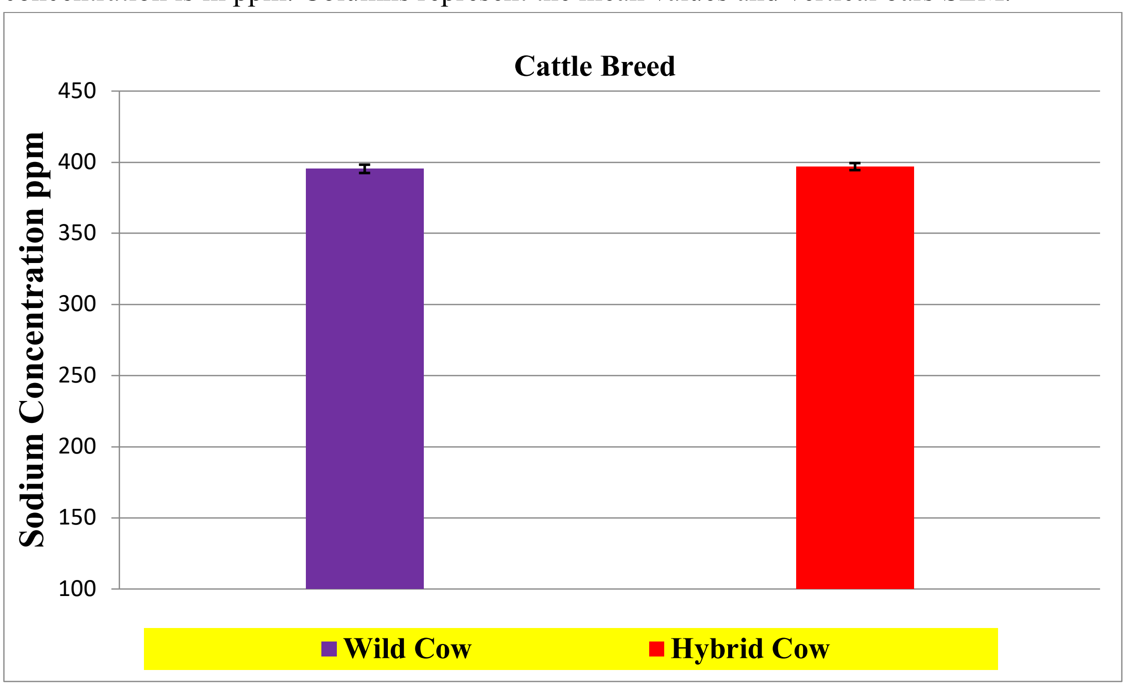


Figure -4: Concentration of potassium in raw milk of wild and hybrid cows. Value of potassium concentration is in ppm. Columns represent the mean values and vertical bars SEM.

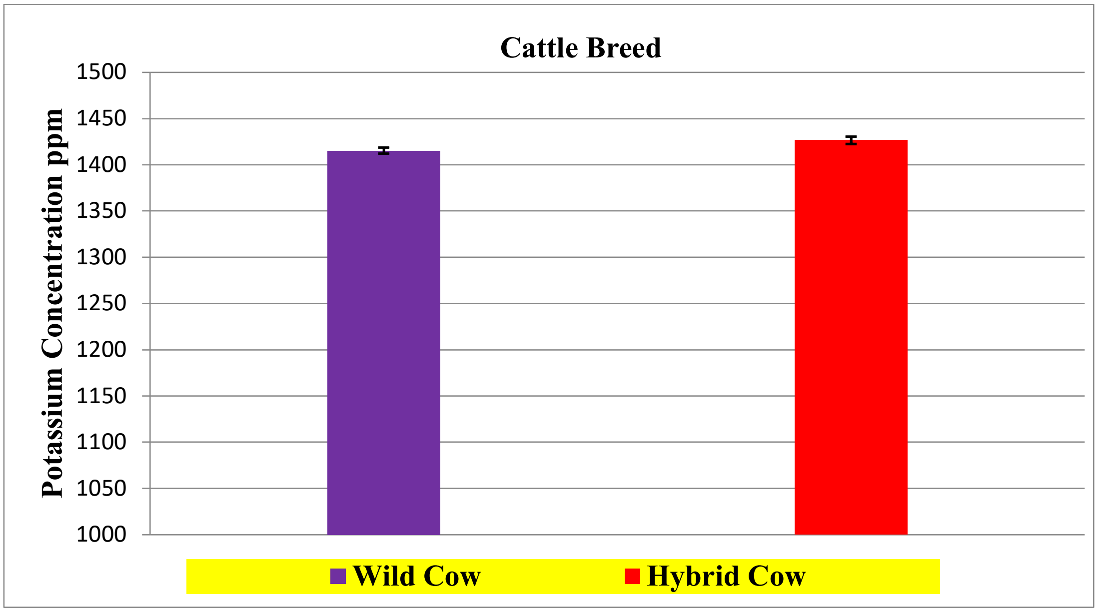


Figure -5: Concentration of iron in raw milk of wild and hybrid cows. Value of iron concentration is in ppm. Columns represent the mean values and vertical bars SEM.

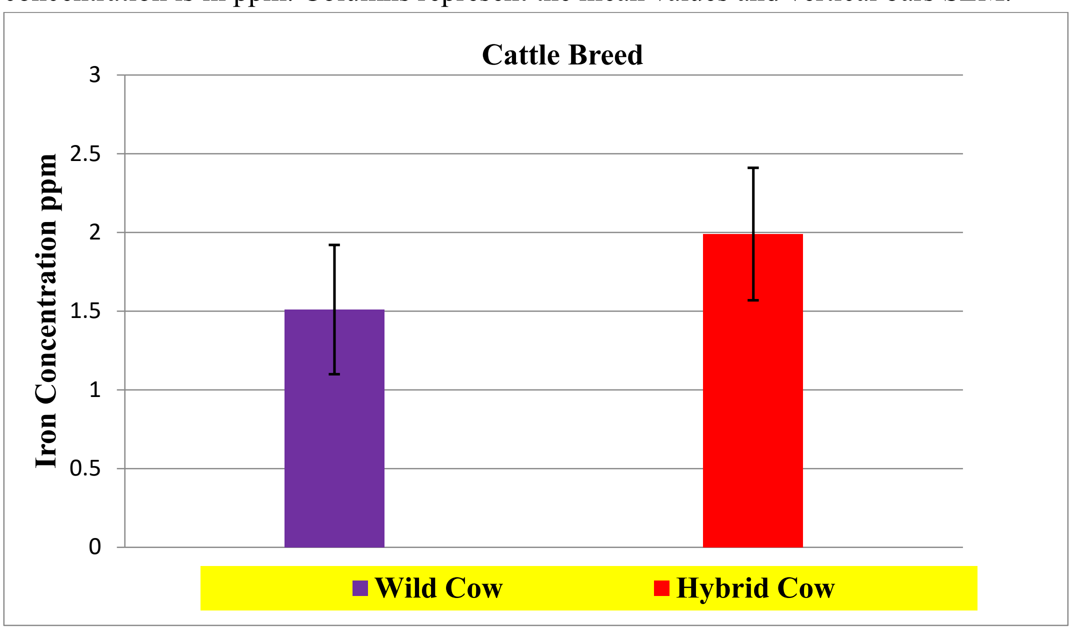
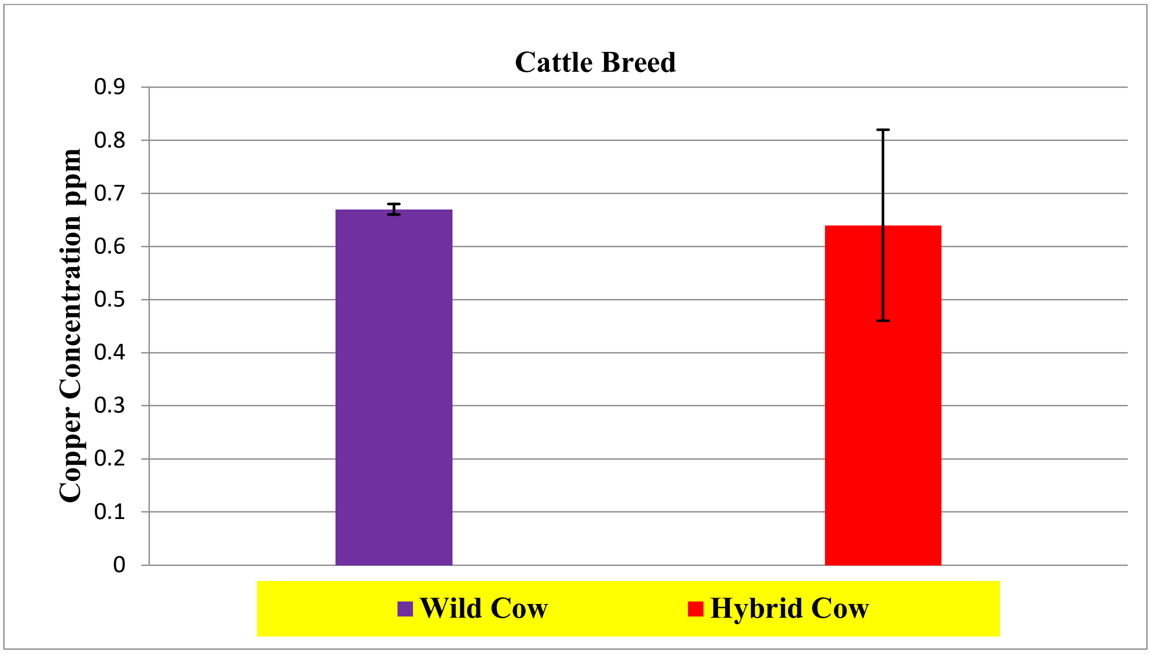


Figure -6: concentration of copper in raw milk of wild and hybrid cows. Value of copper concentration is in ppm. Columns represent the mean values and vertical bars SEM.



Discussion

Ca concentrations in different wild cows were ranging from 1196 ppm to 1278 ppm. The mean ± SEM of calcium was 1229 ± 8.949 ppm. Ca concentration in different hybrid cows was ranging from 1280 ppm to 1330 ppm. The mean ± SEM of calcium was 1304 ± 5.013 ppm. The calcium concentrations of hybrid cows were found little higher as compared to wild cows. Requirements of Calcium in lactating cows are high as compared to non lactating cows. Therefore calcium in the form of dicalcium phosphate or Caco3 should be mixed with the diet of lactating cattle. Milk is rich in calcium and intake of appropriate amount of milk prevents osteoporosis in human (Thomas H.Herdt, 2020).

Mg concentration in different wild cows was ranging from 129 ppm to 142 ppm. The mean ± SEM of Magnesium was 136.3 ± 1.46 ppm. Magnesium concentration in different hybrid cows was ranging from 134 ppm to 150 ppm. The mean ± SEM of Magnesium was 141.7 ± 1.77 ppm. The Magnesium concentration was found very little difference between wild and hybrid cows. Supplement of magnesium is required with diets which are available with high amount in grass forages. Magnesium plays a very important role in the human body and its deficiency causes critical health issues like skeletal muscle loss and metabolic syndrome. Average content of magnesium in cow's milk is 110 mg per litre (Eustina oh and deeth, 2017).

Na concentration in different wild cows was ranging from 382 ppm to 412 ppm. The mean ± SEM of Sodium are 395.3 ± 2.82ppm. Na concentration in different hybrid cows was ranging from 382 ppm to 408 ppm. The mean ± SEM of Sodium is 396.9 ± 2.52ppm. The Sodium concentration was found very little difference between wild and hybrid cows. Sodium usually required to be added as common salt or Nacl. Inadequate intake of sodium reduces the performance of animals. Lactating cows reduces milk production within 1–2 week if the salt is removed from their diets. Supplementary salt is essential during the time of heat stress but excessive salt supplementation may causes udder oedema in calving cows. (Thomas H.Herdt, 2020)

Potassium concentration in different wild cows was ranging from 1395 ppm to 1425 ppm. The mean ± SEM of Potassium are 1415 ± 3.2ppm. Potassium concentration in different hybrid cows was ranging from 1400 ppm to 1439 ppm. The mean ± SEM of Potassium is 1427 ± 4.06 ppm. The Potassium concentration of hybrid cows was found little higher as compared to wild cows. Potassium causes several important biological processes in dairy cows like the maintenance of osmotic potential within the cells, metabolic activities of cell, voluntary and involuntary muscle function, excretory function and transmission of nerve impulses. Appropriate amount of potassium in forage material is required for the good health of dairy cattle. Continuously milking reduces potassium level in dairy cows. (Thomas H.Herdt, 2020).

Iron concentration in different wild cows was ranging from 1.39 ppm to 1.78 ppm. The mean ± SEM of Iron is 1.51 ± 0.04 ppm. Iron concentration in different hybrid cows was ranging from 1.8 ppm to 2.2 ppm. The mean ± SEM of Iron is 1.99 ± 0.04 ppm. The Iron concentration of hybrid cows was found little higher as compared to wild cows. Deficiency of iron is extremely unusual in dairy cattle. Iron deficiency causes anaemia and inflammatory diseases. Suckling calves need extra iron for good health. (Thomas H.Herdt, 2020).

Copper concentration in different wild cows was ranging from 0.6 ppm to 0.71 ppm. The mean ± SEM of Copper is 0.67 ± 0.01ppm. Copper concentration in different hybrid cows was ranging from 0.64 ppm to 0.78 ppm. The mean ± SEM of Copper is 0.71± 0.01 ppm. The Copper concentration was found very little difference between wild and hybrid cows. The suggested concentrations of copper in cow's diets are 10 to15 mg/kg dry matters but Fe, Zn and Ca can hinder the copper accessibility. Deficiency of copper is categorised by hair loss pigmentation, hair loss around the eyes, anaemia, immune suppression and persistent diarrhoea (Thomas H.Herdt 2020).

The trace elements are essential for health, growth and reproduction. They are also essential for a number of functioning components of the immune system. So they contribute to maintain immunity and proper health. They are also very important for functioning of a number of enzymes and protein which are involved in many physiological and biochemical process related to growth, production and reproduction. Hence trace elements affect both the health and production performance of animals (Nileshkumar and Subha, 2018).Trace elements are act as antioxidants (Andrieu, 2008; NRC, 2001) and prevent oxidative stress (Gressley, 2009). Rabiee *et al.,* (2010) reported that trace elements increased conception rates in cattle. Trace elements like Cu, Zn and Fe affect thyroid hormone production in cattle (Abdollahi *et al.,* 2013; Hess *et al.,* 2002 and Nileshkumar and Subha, 2018). Copper regulates progesterone production by luteal cells in cattle (Sales *et al.,* 2011).Iron also regulates ovarian activity (Qian *et al.,* 2001). They are mainly required for reproduction (Rabiee *et al.,* (2010); Ceylan *et al.,* 2008).

Conclusion

The concentration of trace elements like Calcium, Potassium and Iron was higher in hybrid cows in comparison to the wild cows. Whereas the concentration of trace elements like Magnesium, Sodium and Copper was very little difference found in both hybrid and wild cows. This study revealed that both breed of cows contains more or less amount of trace elements especially wild cows contains high concentration of copper in comparison to hybrid cows.

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