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The Comparison of Essential Metals (Fe, Mg, Ca, Zn, Cu, and Mn) in Bali Cattle and Goat Liver

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ABSTRACT

Nutrient metabolism in the liver is greatly influenced by essential metal contents such as iron (Fe), magnesium (Mg), calcium (Ca), zinc (Zn), copper (Cu), and manganese (Mn), which serve as enzyme cofactors. The liver of cattle and goats is beneficial for the metabolism of consumers' bodies since they are widely consumed by the public. Therefore, this research aims to compare essential metal contents such as Fe, Mg, Ca, Zn, Cu, and Mn in the liver of cattle and goats. A total of 30 liver samples were obtained from slaughterhouses in Denpasar City, Bali Province of Indonesia. All cattle liver samples were collected from Bali cattle, while that of goats were a mixture of several breeds. Liver tissue was extracted, and the essential metals were measured using atomic absorption spectrophotometry (AAS). The average essential metal contents in cattle were Fe=79.18±53.96ppm, Mg=86.29±68.05ppm, Ca=80.45±77.87ppm, Zn=53.64±4.92ppm, Cu=24.13±16.87ppm, and Mn=1.29±0.48ppm. Meanwhile, the average results in goats were Fe=57.1718±18.32ppm, Mg=33.81±2.49ppm, Ca=33.77±2.21ppm, and Mn=2.22±0.49ppm. Zn and Cu were not detected in any of the 30 goat liver samples examined. It was discovered that Fe, Mg, Ca, Zn, and Cu in cattle liver are higher than in goat liver, except for Mn. Therefore, it can be concluded that the quality of Bali cattle liver is higher than that of the goat in terms of Fe, Mg, Ca, Zn, and Cu, while the quality of goat liver is better in terms of Mn content.

Key words: Liver, Essential metals, Cattle, Goat.

INTRODUCTION

Cattle and goat meat are the most popular animal protein sources consumed by the community (Besung et al. 2019) To meet their metabolic needs, these ruminants obtain nutrition from available feed and are sometimes given specific supplements in intensive farming. Cattle and goats not only need carbohydrates, proteins, and fats but also require essential metals such as Fe, Mg, Ca, Zn, Cu, and Mn. Non-essential heavy metals including mercury (Hg), lead (Pb), and cadmium (Cd) are not needed in animals' metabolism but can enter their body as contaminants (Hejna et al. 2018). In Suwung Denpasar City, Bali cattle contain lead (Pb) in their blood, liver, spleen, and kidneys (Berata et al. 2017; 2021).

The quality of cattle and goat meat is highly determined by the nutrients they consume, including the content of essential metals. Iron, which is a special protein binding to oxygen is needed by the body to form hemoglobin (Gupta 2014). Magnesium plays an important role in enzymatic reactions since it serves as a cofactor for adenosine triphosphatase and becomes a cell energy source (al-Ghamdi et al. 1994; Khani et al. 2023). Furthermore, this type of essential metal is involved in over 300 enzyme systems that regulate various biochemical reactions including protein synthesis, muscle and nerve function, blood glucose regulation, blood pressure regulation, maintenance of cell membrane integrity, nervous tissue function, neuromuscular, muscle contraction, hormonal secretion, and nutrient metabolism (Laires et al. 2004). Calcium plays a major role in forming the bone and teeth system. The animal body tends to take calcium from the bone to cause osteoporosis when its content is insufficient in food (Pettifor et al. 2010). Furthermore, zinc plays an important role in forming

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materials and cell structures, as well as helping to stabilize cell membranes and DNA. It also acts as an antioxidant for the body and helps Fe forming hemoglobin (Brown et al. 2001; Hegab and Mohamaden 2023).

Copper is another essential metal content that strengthens blood vessels and epithelial cells as well as connective tissues (Osredkar and Sustar 2011). Both animals and humans need manganese (Mn), particularly for the endogenous antioxidant, manganese superoxide dismutase (MnSOD), which prevents oxidative stress in cell mitochondria. Moreover, deficiency or excess of Mn in the body can increase the production of reactive oxygen species (ROS), which can cause metabolic disorders (Li and Yang 2018). Disorders of the liver such as cirrhosis can be triggered by various causes such as interference with essential heavy metals and other nutrients imbalance. In the appropriate amount, this essential heavy metal is needed to compose digestive enzymes such as the production of bilirubin, the metabolism of nutrients, fats, and drugs (Ozougwu 2017).

The structure of the liver in animals varies greatly, but its function is almost the same as that of humans (Madhan and Raju 2014). Meanwhile, the liver is regarded as the main organ responsible for metabolizing various nutrients, including essential metals, and is an important indicator of meat quality. High-quality meat is highly demanded by consumers in terms of health and complete nutritional composition. From a health perspective, meat derived from the liver needs to be examined for pathological changes that are caused by infection and poisoning. Pathological lesions include necrosis and inflammation (Jaishankar et al. 2014). However, common liver pathological disorders are caused by *Fasciola gigantica* infection (Winaya et al. 2020). Pathological disorders can bring about impair optimal metabolism and low-quality meat. Therefore, it is necessary to conduct research to compare essential metal contents such as Fe, Mg, Ca, Zn, Cu, and Mn in the liver of cattle and goats.

MATERIALS AND METHODS

Research Sample

A total of 30 liver samples each from both Bali cattle and goats were obtained from slaughterhouses in Denpasar City. The collection of liver samples of the ruminants was conducted through cross-sectional research, following the recommendations of animal ethics No. B/250/UN.14.2.9/PT.01.04/2022.

The Measurement of Essential Heavy Metal Content

The essential heavy metals, including Fe, Mg, Ca, Zn, Cu, and Mn, in samples of cattle and goat liver, were measured at the Analytical Laboratory of Udayana University, Badung, Bali Province of Indonesia using the Atomic Absorption Spectrometry (AAS) method (Sikiric et al. 2003). Two grams of liver tissue were divided into 1g each for both a positive control and measured sample. This positive control (spiked) was prepared by adding 0.25mL with 1mg/L of a standard solution. The spike was evaporated on a hot plate at a temperature of 100ºC until it dry. Furthermore, the measured sample and spiked were placed in a combustion furnace with half of the surface covered. This furnace temperature was gradually increased by 100ºC every 30min until it reached 450ºC and maintained for 18 hours. The measured sample and spiked were also removed from the combustion furnace and cooled to room temperature. After cooling, 1mL of 65% HNO3 was added and shaken slowly until all ash was dissolved in acid. Furthermore, it was evaporated on a hot plate at a temperature of 100ºC until dry. The measured sample and spiked were returned to the combustion furnace and the temperature was gradually increased by 100ºC every 30min until it reached 450ºC and maintained for 3 hours. After the ash was perfectly formed and white, 5mL of 6M HCl was added, shaken slowly until all ash was dissolved in acid, and then evaporated on a hot plate at 100ºC until it dry. About 10mL of 0.1M HNO3 was also added and cooled to room temperature for 1 hour. The solution was transferred into a 50mL polypropylene volumetric flask and the matrix modifier solution was added up to the mark using 0.1M HNO3. Additionally, the essential heavy metal standard working solutions were prepared for each sample and spiked with a minimum of five concentration points. The standard working solution, sample, and spiked were read on the graphite furnace atomic absorption spectrophotometer at the wavelength corresponding to the metal being tested. Finally, the concentration in µg/g was calculated using the following formula (SNI 2354.5:2011):

$$Concentrate = \frac{(D - E)\ x\ Fp\ x\ V}{W}$$

Description:

D: Concentration of sample in µg/L from AAS reading

E: Concentration of blank sample in µg/L from AAS reading

Fp: Dilution factor

V: Final volume of prepared sample solution (in mL), converted to liters

W: Weight of sample (in grams)

An Independent t-test was done to compare the essential heavy metals content in the liver of cattle and goat.

RESULTS

The result of the heavy metal measurement showed higher variation in Bali cattle than in goats, specifically for Fe, Mg, and Ca. This variation is based on a higher standard error in cattle. The measurement data for cattle (mean±SD) were 79.18±53.96, 86.29±68.05, 80.45±77.87, 53.64±4.92, 24.13±16.87, and 1.29±0.48ppm Fe, Mg, Ca, Zn, and Cu, respectively. Meanwhile, in goats, the results were Fe=57.1718±18.32ppm, Mg=33.81±2.49ppm, Ca=33.77±2.21ppm, Mn=2.22±0.49ppm. Among the 30 goats' liver samples examined, no Zn and Cu were detected. The measurement error (SE) shows higher variation in cattle compared to goats, except for Mn. Table 1 shows the measurement data and indicates the comparison of the levels of essential heavy metals between Bali cattle and goats' livers. Table 1 shows that the level of Fe in Bali cattle liver was significantly higher than in goat ($P<0.05$); Also, Mg, Ca, Zn and Cu level of Bali cattle liver was highly significantly greater than in goat ($P<0.01$); However, the Mn level of Bali cattle liver was significantly lower than goat ($P<0.01$). We also found that goat liver did not contain Zn and Cu.

Table 1: Comparison of the level of essential metals in Bali cattle (n=30) and goat (n=30) liver

Variable (ppm)	Sample	Mean	SD	SEM	T	P
Fe	Cattle	79.1773	53.94628	9.84920	2.116	0.039
	Goat	57.1710	18.31626	3.34408		
Mg	Cattle	86.2882	69.05369	12.60742	4.16	0.000
	Goat	33.8083	2.48850	0.45434		
Ca	Cattle	80.4528	77.87001	14.21705	3.335	0.001
	Goat	32.8683	6.57680	1.20075		
Zn	Cattle	53.3412	3.13721	0.57277	93.128	0.000
	Goat	0.0000	0.00000	0.00000		
Cu	Cattle	25.3026	17.92098	3.27191	7.733	0.000
	Goat	0.0000	0.00000	0.00000		
Mn	Cattle	1.2903	0.48126	0.08787	-7.14	0.000
	Goat	2.2173	0.48783	0.08907		

Ca=Calcium; Cu=Cuprum/copper; Fe=Ferus/iron; Mg= Magnesium; Mn=manganese; N=Total sample; P=Probability; T=T test value; Zn=Zinc.

DISCUSSION

The liver plays a central role in metabolizing all substances that enter the circulatory system. Meanwhile, the structure of the liver varies greatly among animals, its functions are similar to those of the human liver (Madhan and Raju 2014). Its primary functions in both animals and humans are metabolizing nutrients such as carbohydrates, proteins, and fats, detoxifying toxic substances, producing albumin, producing bilirubin, filtering blood from infectious agents, as well as storing vitamins and minerals (Anderson and Frazer 2005). The liver can also be examined to determine the excess or deficiency of required substances, including essential heavy metals. Essential heavy metals become toxic and cause changes in the microscopic structure of the liver tissue when their levels are excessive. Meanwhile, pathological changes in the liver due to toxicity can result in congestion, bleeding, inflammation, necrosis, and fibrosis (Jaishankar et al. 2014). Pathological liver disorders caused by infectious agents or poisoning can also affect the essential metal content of the liver (Ozougwu 2017).

The anatomical and histological structures of the livers of cattle and goats are very similar, both in terms of the shape of the lobules and the hepatocytes (Madhan and Raju 2014). However, there are variations in metabolism in cattle and goat liver (Ozougwu 2017). We prove this by looking at the results of this study, namely the differences in the content of essential heavy metals.

The average Fe content in Bali cattle liver was 79.18±53.96ppm, while that of the goat liver was 57.1718±18.32ppm in the present study. In Fe, the variation in cattle liver was higher than in goats, indicating that consuming cattle liver is not always better than consuming that of the goat. This is also related to the variation in feed as a source of Fe for the body's needs in cattle. Both cattle and goats obtain Fe from the grass they consume, and the content of this essential metal in the soil determines the one in the grass (Jones-Lee and Lee 2005). In the body, Fe plays a vital role as a component of hemoglobin and myoglobin, which transfer oxygen into cells. It is estimated that 70% of the Fe is found in the form of hemoglobin. Iron is abundant in organs involved in red blood cell formation (hematopoiesis), such as the bone marrow, liver, and spleen. Furthermore, Fe plays a role in the function of electron transfer enzyme chains such as cytochrome oxidase, ferredoxin, myeloperoxidase, catalase, succinate dehydrogenase, and cytochrome P-450. Iron absorption in the small intestine occurs more in the form of Fe^{2+} (ferrous) than Fe^{3+} (ferric). Fe binds to chelators such as histidine, mucin, or fructose in the small intestine mucosa. After absorption, Fe in the bloodstream binds to transferrin. Approximately 60% of Fe is found in hemoglobin (Gupta 2014).

Iron is stored in the body as ferritin, and when there is an excess amount, it is stored as hemosiderin. Fe is processed into protoporphyrin and then into heme in reticulocytes. Iron metabolism can be disrupted when there is an excess of Zinc exceeding 23-63ppm Zn (DM) in cows. The therapeutic effect of Cu can reduce iron metabolism in the liver and hemoglobin (Anderson and Frazer 2005).

The Mg content in cattle liver was 86.29±68.05ppm, while that of the goat was 33.81±2.49ppm in the present study. However, this content in cows is generally reported to be around 180-190ppm (Goglia 2022). Mg plays an important role in many enzymatic reactions involved in energy metabolism, which serves as a cofactor of adenosine triphosphatase (al-Ghamdi et al. 1994). It is involved in more than 300 enzyme systems that regulate various biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose and blood pressure regulation, maintenance of cell membrane integrity, neural tissue function, neuromuscular function, muscle contraction, hormonal secretion, as well as nutrients' metabolism (Laires et al. 2004). Magnesium is important for the absorption of vitamins and minerals, such as sodium, calcium, potassium, and phosphorus. Generally, mineral absorption takes place in the small intestine to ensure that toxins are removed from the body. Balanced magnesium intake also helps to activate vitamin D that is stored in the body. In mammals, magnesium plays a vital role in maintaining the liver from disturbances such as cirrhosis, alcoholic liver disease (ALD), liver cancer, and viral hepatitis (Liu et al. 2019). It also serves as a cofactor, and an imbalance of magnesium can cause pathological disorders. Magnesium deficiency (Hypomagnesemia) can cause various disorders including alcohol disease, diabetes, cardiovascular disease, eclampsia, chronic alcoholism, and cataract pathogenesis in the eye (Laires et al. 2004; Agarwal et al. 2012). Cattle with hypomagnesemia during pregnancy can develop grass tetany disease characterized by animal collapse. Conversely, excess Mg can cause diarrhea and disrupt Ca and phosphorus metabolism (Pinotti et al. 2021).

The measurement results for Ca content in Bali cattle showed an average of Ca=80.45±77.87ppm, while in goat liver, it was 33.77±2.21ppm in the present study. This indicates that cattle liver is a better source of Ca. When the Ca is not enough, the body tends to extract it from the bones, which can lead to osteoporosis (Pettifor et al. 2010). About 99% of the Ca in animal and human bodies is stored in bones and teeth. Ca is essential for bone development, growth, and maintenance. After bone growth stops, it helps to preserve and slow down bone density loss. Ca regulates various nerve functions, including neurotransmitter synthesis and release, nerve stimulation, as well as phosphorylation. It is also involved in long-term processes, such as memory cell formation in the brain. Although the general mechanism of the body's response to Ca is in the absorption in the intestine, storage in bone, and reabsorption in the kidney, Ca metabolism occurs in mitochondrial cells, specifically in hepatocytes (Alonso et al. 2006; Rizzuto et al. 2012). Ca in the cytosol, mitochondria, and nucleus are the same in the resting state but differs in activity (Peacock 2010). The Ion Ca2+ plays a crucial role in triggering various physiological and pathophysiological processes in cells, including cancer (Dejos et al. 2020).

In the present study, the average Zn content in Bali cattle liver was 53.64±4.92ppm, while none was found in goats examined. From the perspective of Zn, Bali cattle liver is better than that of the goat. It has been reported that cattle liver generally has a higher Zn content than those of goats and sheep (Abdelbasset et al. 2014). Zn plays a critical role in cell development and differentiation. The body requires Zn as a component and structure of cells, as well as for stabilizing cell membranes and DNA, making it an antioxidant and assisting in the formation of hemoglobin through the Fe process (Brown et al. 2001). It is vital for cell proliferation and differentiation, particularly in the regulation of DNA synthesis and cell mitosis processes. In some metabolic processes, Zn binds to metallothionein proteins. Additionally, Zn can function as a modulator of cell recognition, a messenger carrier of metabolic activity from protein kinase and phosphatase, as well as stimulate or inhibit transcription factor activity (Beyersmann and Haase 2001). Zn not only aids in improving the immune system but also the digestive and nutrient absorption systems. Its other functions include assisting in the formation, storage, and release of insulin from the pancreas. Zn plays a significant role in the absorption and metabolism of nutrients. It can help to treat diarrhea in children, chronic hepatitis, shigellosis, tuberculosis, leprosy, pneumonia, lower respiratory tract infections, flu, and leishmaniasis, as well as prevent blindness (Prasad 2009).

These diseases can be prevented when the body's Zn requirement is met through the consumption of cattle or goat meat. Zn deficiency can be caused by various liver diseases, including alcoholic liver disease and viral liver disease. Its deficiency can lead to skin and hair disorders, increase hepatocyte apoptosis, immune disorders, and impaired wound healing. In humans, Zn deficiency is associated with atherosclerosis, malignancy, neurological disorders, degenerative diseases, age-related diseases, autoimmune diseases, and Wilson's disease. Supplementation with Zn can enhance the digestive barrier, strengthen immunity, and reduce oxidative stress in body cells. Zn is more strongly bound to metallothionein (MT) in the liver than copper (Cu), indicating that it plays a more important role in the liver than Cu. Furthermore, the role of Zn and Zn-MT is reported to protect cells from apoptosis processes mediated by oxidative stress by Cu and Fe (Formigari et al. 2007).

The average Cu content in Bali cattle liver was 24.13±16.87ppm, while none was detected in that of the goat in the present study. This result suggested that Cu in cattle is better than in goats. The concentration of Cu in the liver is typically low because ruminant liver has a limited ability to accumulate Cu. Research conducted in Spain showed that the liver had the highest Cu concentration (34.3ppm) compared to other organs in cattle. Similarly, in Moroccan cattle, Cu was found to be highest in liver tissue. Cu is required by the body in various enzymatic functions, strengthening of blood vessels, and the development of epithelial and connective tissues. Cu is also essential for the formation of collagen and elastin, proteins that are vital for connective tissues, skin, nails, and hair. Insufficient intake of Cu can lead to joint problems, as the body is unable to repair damaged connective tissue. Moreover, inadequate collagen production may result in the skin becoming more vulnerable to premature aging and losing its structural and strength support. Cu acts as a catalyst in the formation of reactive oxygen species, which can damage DNA or alter deoxyribose, resulting in cell carcinogenesis (Pitropovska et al. 2014).

The Mn content in goat liver was significantly higher than in Bali cattle in the present study. This also shows the superiority of goat liver nutrition. The role of Mn in metabolism is closely related to antioxidants, namely binding to the oxidative stress factor superoxide dismutase to form MnSOD (Li and Yang 2018). These data are in accordance with previous publications where it was stated that the distribution of Mn in various body tissues of goats can be found in the liver, kidney, heart, brain, and hair. Indeed, the highest levels of Mn were reported in the liver, so the recommendation to detect it is accurate if the liver and hair are examined rather than in the blood (Sikiric et al. 2003).

Conclusion

In terms of the contents of Fe, Mg, Ca, Zn, and Cu, Bali cattle liver was superior to that of goats, whereas goat liver is better in terms of Mn. All of the samples of cattle liver were obtained from Bali cattle, and their essential metal contents were 79.18±53.96, 86.29±68.05, 80.45±77.87, 53.64±4.92, 24.13±16.87, and 1.29±0.48ppm of Fe, Mg, Ca, Zn, and Cu, respectively.

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Authors contribution

All authors were actively involved in different responsibilities. I Ketut Berata, I Made Kardena, Ida Bagus Oka Winaya, and Kadek Karang Agustina: preparing research proposal and completion of the manuscript; I Ketut Berata, Kadek Karang Agustina and Ni Luh Watiniasih: statistical analyses, I Ketut Berata: laboratory testing and analyses.

Conflict of Interest: None.

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