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IMPLEMENTATION OF ESSENTIAL BIOSTIMULATOR TECHNOLOGY IN SUPPLEMENTARY FEED AS A MIXTURE OF REINFORCING FEED ON HAEMATOLOGICAL AND NUTRIENT PROFILES OF BEEF CATTLE SERUM (CASE STUDY OF BEEF CATTLE FATTENING ON PEOPLE'S LIVESTOCK)

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ABSTRACT

The national meat self-sufficiency program in Indonesia aims to increase the cattle population to meet the country's meat consumption. Beer dregs are a potential alternative feed supplement, as they contain high crude protein and soluble carbohydrates that can affect the rumen microbial population. In addition, essential oil biostimulators derived from citronella are added to the feed as a mixture of reinforcing feed to support cattle health and productivity. This study investigates the effect of essential biostimulators in supplementary feed as a mixture of reinforcing feed on the haematological and nutrient profiles of beef cattle serum, using the case study of beef cattle fattening in People's Livestock. Results showed that the addition of citronella essential oil improved nutrient digestibility and rumen health, and the combination of beer dregs and essential biostimulators positively impacted the haematological and nutrient profiles of beef cattle serum.

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Keywords: Beer dregs, Essential oil biostimulators, Cattle, Serum, Haematology, Nutrient profiles, Feed supplement, Rumen microbial population, Nutrient digestibility.

INTRODUCTION

The national meat self-sufficiency program is an activity to increase the domestic livestock population, especially cattle, to meet the national meat consumption of 696,956 tons in 2021 with a per capita consumption calculation of 2.56 kg per year. The success of this program has implications for reducing the percentage of imports of live cattle to only 502,000 heads and frozen meat to 284,277 tons in 2021 so that in the future, Indonesia will reach the stage of self-sufficiency [1].

The beef cattle fattening business belongs to the Harapan Jaya III Farmers Group with Ministry of Law and Human Rights' Decree Number AHU-0015791.AH.01.07. 2016 has the potential to be developed for a meat self-sufficiency program. The location is in Kedawung Selatan Hamlet, Bicak Village, Trowulan District, Mojokerto Regency, adjacent to the beer factory PT Multi Bintang Indonesia Tbk. This beer producer produces waste in the form of beer dregs and liquid containing mold left over from fermentation.

Beer dregs can be an alternative constituent of feed supplement for cattle. The high value of crude protein and soluble carbohydrates contained in beer dregs can affect the rumen microbial population. Bacteria and protozoa hydrolyze protein from the feed to produce amino acids, and then deamination occurs to produce ammonia. Meanwhile, carbohydrates from the feed are fermented by rumen microbes into volatile fatty acids (VFA), which are used as an energy source for microbes and the host (livestock) [2]. The results of the proximate analysis showed that the beer dregs had high nutrition, namely 27.5% crude protein; 7.5% crude fat; 10.5% crude fiber; 41.1% extract material without nitrogen; 0.29% calcium (Ca); 0.48% phosphorus (P), total digestible nutrient (TDN) 69.89%.

Feeding beer dregs up to 40% can increase consumption of Crude Protein (PK), Total Digestible Nutrient (TDN), and Crude Fiber (SK) but does not affect consumption of BK, PBBH, and feed conversion for local male sheep [3]. The addition of beer dregs of 19% in local male sheep provides higher protein digestibility than the addition of 9% because the PK of the feed increases, so the availability of ammonia as Non-Protein Nitrogen (NPN) to be converted into body protein also increases. There is also an increase in the population of rumen microbes and fermentation processes that increase PK's digestibility [4]. In beef cattle, feeding beer dregs at a dose of 9 kg gave the highest body weight gain after 30 days of administration. There was a difference in body weight of 77.89% compared to controls [5].

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In addition to providing feed according to their needs, beef cattle also need an essential oil bio stimulator from citronella. Citronella is added to the premix as a mixture of reinforcing feed to support livestock health so that it can achieve maximum productivity, according to its genetic potential. The chemical components contained in citronella include citronellal (32-45 percent), geraniol (12-18 percent) and citronella (12-18 percent) [6]. Essential oils in lemongrass, which function as antioxidants, include flavonoids, citronellal, citral, and phenolics [7]. Essential oils are also helpful as antibacterial, antifungal, anticancer [8], and anthelmintic because they can reduce the number of adult *H. contortus* worms in sheep, with an efficacy of up to 66.4% when compared to the control group [9].

The essential oil contained in citronella leaf extract, *Cymbopogon citratus* (*C. citratus*) has three times stronger anthelmintic activity than *Ocimmum gratissimum* (*O. gratissimum*) against adult *Haemonchus placei* (*H. placei*) in vitro. Phytochemical analysis of *C. citratus* extract showed the content of active substances, including alkaloids, tannins, steroids, saponins, terpenoids, and flavonoids [10]. Adding lemongrass does not affect feed consumption but improves nutrient digestibility and rumen health [11].

Blood has a vital and complex role because it determines the continuity of normal physiological processes in the animal's body so that livestock productivity is optimally achieved. Blood consists of cellular components, namely erythrocytes, leukocytes and platelets, and plasma fluid.

The ration is essential for blood metabolism because it needs protein, vitamins, and minerals to form red blood cells. An erythrocyte examination was carried out to determine the state of anemia and polycythemia [12].

The process of erythropoiesis needs the role of vitamins and minerals, especially vitamin B6 and iron which are needed in the enzymatic process of heme synthesis. Copper in the form of ceruloplasmin is essential in releasing iron from tissues to plasma. Cobalt is important in synthesizing vitamin B12 by ruminants [13].

This study raises the effect of essential oil bio stimulators in feed supplements as a mixture of booster feed on beef cattle serum's hematological and nutrient profiles.

MATERIALS AND METHODS

Treatment Feeds

The treatment feed was homemade, containing 40% beer dregs and 60% concentrate, given for one month, with the nutritional composition presented in Table 1.

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Table 1: Nutritional Composition of Treatment Feed

No	Nutrients	Content (%)
1.	Moisture content	11.42
2.	Dry matter (DM)	88.58
3.	Ashes	15.89
4.	Proteins	16.27
5.	Crude Fat	3.97
6.	Crude Fiber	21.65

The premix added to the booster feed is branded "Premix Unggul," containing minerals, vitamins, and essential oils (Table 2). Lemongrass essential oil is produced by PT Pemalang Agro Wangi, Bogor, with 100% content and added to the premix as much as 2%.

Table 2: Composition of Minerals, Vitamins, and essential oils in premixes

No	Mineral	Content
1.	Calcium (g)	328
2.	Phosphor (g)	149
3.	Magnesium (g)	12.5
4.	Selenium (g)	8.7
5.	Iodine (g)	8.9
6.	Zinc (g)	18
7.	Cobalt (g)	8.69
8.	Vitamin A (IU)	320,000
9.	Vitamin E (IU)	250

Animals

This research is a case study on smallholder beef cattle farming, a semi-intensive pattern belonging to the Harapan Jaya III Farmers Group, Mojokerto, East Java. Experimental cattle were of the Simental type, consisting of 11 females and 3 males, ages ranging from 1 to 1.5 years. Cattle in lean condition, body weight gain is still less than 500 grams per head per day. Forage feed in the form of rice straw, without concentrate, never given deworming before, open-type cage, ground floor, not cemented.

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Blood Collection and Evaluation of Haematological Profile and Serum Nutrient

Blood samples were collected aseptically from the jugular vein with a sterile needle into a 5 ml vacutainer tube, then 2.5 ml of blood was put into a tube containing EDTA for examination of the hematological profile, and the remaining 2.5 ml of blood was put into a tube containing heparin for serum nutrient examination. The hematological profiles tested included the number of leukocytes, monocytes, lymphocytes, granulocytes, platelets, erythrocytes, hemoglobin levels, hematocrit and erythrocyte indices which included Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Volume (MCV). These parameters were analyzed using a Rayto RT 7600 Hematology Analyzer. Serum nutrients tested included triglycerides, cholesterol, glucose, and total protein. This examination uses the Ubio-iChem-535 blood chemistry analyzer. Blood tests were carried out twice, namely before and after giving the treatment feed.

Data Analysis

The research data were tabulated in the Excel program, then analyzed using quantitative descriptive analysis by comparing the mean hematological and nutrient serum values before and after the feeding treatment.

RESULTS

The average number of leukocytes, lymphocytes, and granulocytes before and after feeding the treatment feed was above the normal range [14]. The mean of these cells sequentially before treatment was $15.10\times10^3/\mu L$, $7.45\times10^3/\mu L$, and $7.06\times10^3/\mu L$. After treatment was $17.46\times10^3/\mu L$, $9.48\times10^3/\mu L$ and $0.66\times10^3/\mu L$. Leukocytosis, lymphocytosis, and granulocytosis after feeding were higher than before, while the number of monocytes before and after treatment was within the normal range. The number of erythrocytes, hemoglobin, hematocrit, MCHC, MCH, and MCV, both before and after treatment, were below the normal range [14]. Administration of beer dregs and essential oils did not change the anemic condition of the experimental cows. Hematological profile data on experimental cattle, between before and after treatment feeding, (Table 3).

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Table 3: Hematological profile of experimental cows, before and after treatment feeding

Parameter	Unit	Range (Majory and Brooks, 2022)	Mean Before	Mean After
Leukocyte	10^3/µL	4.40 – 10.80	15.10±4.28	17.46±9.18
Lymphocyte	10^3/µL	1.80 – 4.90	7.45±3.95	9.84±5.71
Monocyte	10^3/µL	0.30 – 1.20	0.59±0.18	0.66±0.44
Granulocyte	10^3/µL	0.80 – 5.00	7.06±2.04	6.96±4.18
Erythrocyte	10^6/µL	4.80 – 7.60	4.81±0.66	4.68±0.64
Hemoglobin (Hb)	g/dL	8.20 – 13.00	3.92±1.45	3.57±1.27
Hematocrit	%	24.00 – 39.00	18.75±2.55	18.68±2.86
MCHC	g/dL	32.40 – 35.80	20.41±5.99	18.64±4.90
MCH	pg	14.30 – 19.67	7.92±2.15	7.43±1.96
MCV	fL	41.20 – 58.70	39.16±4.18	40.06±4.49

On examination of nutrient serum (Table 4), triglyceride and cholesterol levels before and after feeding were within normal limits [15], and glucose levels before treatment were within normal limits. However, after treatment, there was a slight increase. Total serum protein before treatment was below normal. On the other hand, after treatment, it was within normal limits.

Table 4: Serum Nutrients of Experimental Cattle before and after treatment feeding

Parameter	Normal Unit	Range (Chirkena et al., 2016)	Mean Before	Mean After
Triglyceride	mg/dl	8.85-36.28	30.43±19.12	36.71±15.72
Cholesterol	mg/dl	36.00-111.60	112.64±53.44	96.36±12.83
Glucose	mg/dl	45.00-69.00	54.21±15.17	71.21±19.93
Total Protein	g/dl	67.00-75.00	50.15±24.37	70.07±2.60

DISCUSSION

Regarding leukocytosis, there is an indication that an infection or inflammation had occurred before being given the treated feed. The cow's body defenses were very low due to poor nutrition. The cows were only given grass, not given concentrate, so the cows were suspected of

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having a deficiency of protein, energy, vitamins, and minerals. Leukocytes are blood components that play a role in the body's defense system to fight infection [16].

Leukocytosis, which increased after the feeding treatment, showed that administering beer dregs and essential oil from citronella was a good combination of a booster feed and a balanced supplement to protect the body against infection. White blood cells protect the body from infection utilizing phagocytosis, synthesis of antibody molecules, destruction of bacteria, cleaning of cell remnants in inflamed tissues, and protecting the infected area [17]. Essential oils act as antibacterial, antifungal, and anticancer [8].

Granulocytosis before and after treatment feeding may be in the form of neutrophilia or eosinophilia, which is caused by bacterial or worm infection. Eosinophilia is the body's response to worm infections [18]. In this study, eosinophilia is thought to be due to malnutrition in cattle so that the body's defense system becomes weak, then worm infections quickly occur. Eosinophilia also occurs in cattle that experience intestinal villi proliferation, a mild inflammatory reaction with infiltration of lymphocytes, plasma cells, and eosinophils in the lamina propria of epithelial cells due to various stages of the coccidian life cycle [19,20]. Granulocytosis, which was higher after given treatment feed than before, was thought to be due to the rainy season during the study, which supported cattle being easily infected with worms.

Lymphocytosis after treatment feeding is thought to occur as a convalescent phase of infectious diseases during chronic antigenic stimulation due to infectious agents, neoplasia, and hypoadrenocorticism [21].

The decrease in the number of erythrocytes before the feeding treatment was thought to be due to poor nutrition in the cows, only given grass, without giving concentrate, so it is very likely that there is a deficiency of energy, vitamins E, B12, iron, and folate which are essential for the process of erythropoiesis. Energy consumption determines the high and the low number of erythrocytes in cows that are only fed 100% forage, without concentrate, causing low, easily digestible carbohydrates consumed [22]. As an easily digestible energy source, the concentrate will produce high levels of propionic acid, which is very important as an erythrocyte precursor. The addition of beer dregs booster feed and premix containing essential oils for 30 days has not improved the anemia.

Hemoglobin is a red pigment, an iron-containing complex protein that serves as a carrier for oxygen molecules. The low hemoglobin value in cattle is thought to be related to the low number of erythrocytes [16,23].

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Several factors are thought to have contributed to cases of cattle anemia before feeding treatment, namely feed management, cage, environment, and season. The breeders provide modest feed, only in the form of field grass, without supplementing feed, so it does not meet the needs in quantity and quality. Cages with a dirt floor, without cement and urinary outlet, are very suitable for developing worms and protozoa.

Nutritional deficiencies cause the immune system to decrease, making the body susceptible to infections, including coccidiosis. For this reason, the prevalence rate of *Eimeria* in animals kept in ventilated cages was lower (46.84%) than in non-ventilated cages (50.69%) [24]. Cages with cemented floors and urine outlets had a lower prevalence (42.23%) than cages with non-cemented floors and no urinary outlets (54.16%). The prevalence rate of intestinal helminthiasis in beef cattle in the Lamongan Regency is 24%, which is high [25]. This is due to the rainy season at the time of sampling. The high humidity, the amount of standing water in the sampling area, and the number of plants around the cages help the various types of worms continue their life cycle.

Chronic liver fluke infection is the most common form of liver fluke infection in cattle. It was characterized by loss of appetite, anemia, and hypoalbuminemia [26]. This can lead to lean animals and decreased overall production due to reduced food intake. Furthermore, the prevalence of liver fluke is higher in the rainy season than in the dry season [27]. The prevalence of *D. hospes*, *F. gigantica*, and mixed infection was 52.56%, 12.82%, and 5.13%, respectively, during the dry season, while 58.54%, 20.73%, and 13.41% during the rainy season.

Another cause of anemia is the season. This research was conducted during the rainy season. Cases of anemia in goats based on season [28], were highest in the rainy season (50.00%, n = 40) followed by winter (27.50%, n = 22) and summer (22.50%, n = 18). The highest average EPG value was recorded in October (855.56 ± 128.30), while the lowest value was obtained in January (275.00 ± 87.02) [27]. Higher prevalence of anemia occurs during the rainy season, caused by higher gastrointestinal parasite infections related to climatic conditions, namely, humidity, and temperature, which support the growth and development of infective larvae in grazing areas in that season [29].

Low PCV, hemoglobin, erythrocyte values, and increased leukocyte count may indicate nematode and liver fluke infections. Hematocrit, Hb, total red blood cell, and total white blood cell values are important indices in monitoring helminthiasis. EPG negatively correlates with hematocrit and hemoglobin values [27].

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The cause of anemia is thought to be related to worm infection, as nematodiosis and liver worm infection was found to be associated with anemia and leukocytosis [27]. The average value of Packed Cell Volume (PCV), hemoglobin concentration, total erythrocyte count, MCV, and MCH was lower in worm-positive animals compared to worm-negative animals.

Adding beer dregs supplement feed and premix containing essential oils required a longer time. There need to be more than thirty days to improve the hematological profile of experimental bovine.

Total bovine serum protein after administration of beer dregs showed normal levels. This result was related to the high PK content and digestible carbohydrates in the beer dregs, thereby increasing the availability of NPN as a nitrogen source to increase the microbial population and energy for rumen microbes, further increasing PK, TDN, and SK digestibility [2,3,4].

CONCLUSION

Based on the results of the study, it was concluded that the addition of beer dregs supplement feed and essential oil from citronella in beef cattle did not affect the anemia condition that was occurring in experimental cows reared in a semi-intensive manner. However, it was able to maintain triglyceride, cholesterol, and glucose levels to remain normal and improve total serum protein blood levels to normal. Supplement feed containing 40% beer dregs and essential oil from citronella 2% can be applied to intensive beef cattle fattening programs with quality forage and application of adequate biosecurity. So that maximum daily body weight gain is achieved according to their genetic potential.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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