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Cholesterol and Blood Profile of Bali Cattle Fed *Chromolaena odorata* Weed with Rice Straw as Basal Feed

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Abstract: This study aims to determine the effect of the level of *C. odorata* (CO) silage-based concentrate on the blood profiles of Bali cattle. Three male Bali cattle (initial 2 years old with CV = 19,28%) were randomly assigned to one of three meals: rice straw and concentrates containing *C.odorata* silage 30% (RCO_{30}) or *C.odorata* silage 40% (RCO_{40}) or *C.odorata* silage 50% (RCO_{50}), respectively, using a 3 x 3 Latin Square Design. The was no significant effect between treatments (P> 0.05) on blood cholesterol (195.74-220.49 mg/dL), blood urea (48.41-49.44 mg/dL), total blood plasma (6.08-6.93 g/dL), blood haemoglobin (Hb; 18.18-18.92 g/dL), hematocrit levels (45.56-53.03%), erythrocytes (7.06-8.24 106/µl), and leucocytes (8.56-8.84/µl). Blood glucose levels, however, differed significantly (P<0.05) between treatment. Feeding Bali cattle with different levels of *C.odorata*-based concentrate resulted in the same effect on all blood parameters except blood glucose. Therefore, it is recommended that the use of *C.odorata* in a ration of Bali cattle can be up to 50%.

Keywords: Bali cattle; Blood profile; *chromolaena odorata;* Rice straw; Silage

Introduction

Although the contribution is high and plays an important role as savings, cash, or assets in the livelihoods of smallholders in East Nusa Tenggara Province, Indonesia, the productivity and reproductive performance of Bali cattle is still low. This problem is mainly due to the low quantity and quality of feed available throughout the year. As a result, the mortality rate of high birth calves is 24.1 – 51.2% during the dry season (Jelantik et al., 2008) and the animal's weight loss is 36.91% (Mullik & Jelantik, 2009). Therefore, finding alternative feeds that are nutritious and available all year round is very important. Chromolena odorata (*C. odorata*) is a Siamese weed that invades most grazing lands in the tropics. This weed has been reported to have a high crude protein content of about 13%-35% (Mullik, 2007; Oematan et al., 2020; Oematan, 2023) and high biomass production (around 70 tons BK/ha/yr) (M. L. Mulik et al., 2015), protein score is 88.2% (Apori *et al.*, 2000; M. Ngozi *et al.*, 2009; Oematan *et al.*, 2020) which are categorized as very high, which can potentially be used as protein supplementation for ruminant animals. However, the use of C. odorata as animal feed is hindered by anti-nutritional factors (Ngozi *et al.*, 2009; Oematan *et al*, 2023). Conversely, the organic biomass of this plant also has various anti-nutrient compoundsphenolics, alkanolides, and terpernoits (Singh, 2022). These secondary metabolites can be reduced by the bio-fermentation process (Ridla et al., 2016; Mulik, 2016) before being used as a source of ruminant animal feed (Bira, 2016)

One of the strategies to reduce this anti-nutritional issue is through making silage. However, silage production's good quality depends on its carbon-nitrogen (C/N) ratio. (Mulik (2016) found that the best C/N ratio in the bio-fermentation of *C. odorata* was 30 %.

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Oematan *et al*., (2023) showed that the bio-fermentation of *C. odorata* using a carbohydrate source of rice straw flour with a C/N30 ratio obtained the best results on feed composition. Therefore, adjusting the C/N ratio is vital in making silage *C. odorata* because, naturally, the C/N ratio in *C. odorata* is only about 14.9, so it can produce poor bio-fermentation.

Blood metabolites such as blood glucose, blood plasma protein, and blood urea levels, they are related to nutritional status and cattle productivity. Supply of glucose and protein has a close relationship with various intakes and the physiological state of cattle. The concentrations of blood glucose are high whenever consumption of energy is high. In addition, urea levels in the blood can be used as an index of protein status and usage. However, information regarding *C. odorata* utilisation feed on Bali cattle is limited. Therefore, In this paper, we will report on the effect of different concentrations of *C. odorata*-based concentrates on the ratio of Bali cattle. The feed obtained is expected to be an alternative feed for fattening Bali cattle.

Method

Location of study

The present study was conducted at the Faculty of Animal Science, Marine and Fisheries field laboratory, Universitas Nusa Cendana Kupang, Indonesia

Animals, diet and experimental design

Three male Bali cattle (initial BW = 108 - 160 kg with cv 19,28%; 2 years old) were randomly assigned to one of three meals: rice straw and concentrates containing *C.odorata* silage 30% (RCO_{30}) or *C.odorata* silage 40% (RCO_{40}) or *C.odorata* silage 50% (RCO_{50}, respectively, using a 3 x 3 Latin Square Design. Each cow was housed in individual pens within a covered cattle house facility with access to water at all times. During each period, all cows were fed rice straw ad libitum, and a 2% BW (DM as fed) concentrate diet. The concentrates consisted of *C.odorata* silage, corn meal, pollard, rice bran, salt, and premix, formulated with 16% ME. Feed was provided to the animals twice a day. Each treatment period lasted for 28 days and consisted of 14 days adaptation period and seven days of sample collection.

Variables and measurement

Blood samples from each cow were collected at the end of each study period via jugular vein using vacutainer tubes containing EDTA 4 hours after the morning meal. The samples were immediately placed on ice and brought to the laboratory for later analysis. Blood samples were then analysed for blood glucose, blood urea nitrogen, total cholesterol, total protein plasma, haemoglobin, haematocrit, erythrocyte and leucocyte using spectrophotometric .

Statistical analysis

Data obtained in this study were statistically analysed using General Linear Model (GLM) procedure using SPSS 25. Duncan's multiple range tests within the SPSS program were conducted to examine the degree of significance among means. The significant difference was set at $P < 0.05$ (Morgan et al., 2019).

Table 1. The composition of the reseacch rations.

Feed Ingredients (%)	Rations		
	RCO_{30}	RCO_{40}	RCO_{50}
Rice straw (1/3 part of the body weight)	ad libitum	ad libitum	ad libitum
Concentrate (2/3 part of the body weight) :			
Silage flour *C. odorata*	30	40	50
Wheat / Pollard bran	47	31	14
Rice bran	12	18	10
Milled corn	10	10	25
Salt	0.50	0.50	0.50
Minerals + vitamins (premix)	0.50	0.50	0.50
Amount	100	100	100
Crude protein content	18	18	18

Result and Discussion

Effect of Treatment on Blood Cholesterol Levels

Cholesterol is found in muscle and adipose tissue because it is an essential component of cell membranes and can be stored as cholesterol esters in lipids. Differences in cholesterol content between species are generally caused by cholesterol absorption and biosynthesis, lipoprotein metabolism, diet, genetic variation, subcutaneous and intramuscular fat and body weight (Dessi and Batetta, 2003). Cholesterol levels in this study varied between 195,74 - 220,49 mg/dL. The result of this study was higher than that reported by Syahrir *et al.*, (2010) when mulberry leaf-based concentrate was offered to Bali cattle which is about 207.38 mg/dL vs 168 mg/dL. Similarly, it was lower compared to the results of Mitruka & Rawnsley (1977) on Bali cattle which is between 207.38 mg/dL vs 125 mg/dL. However, the cholesterol level was lower when compared to cholesterol level in dairy cows, about

207.38/dL vs 227.8 mg/100 ml, and the results were still higher for camels, namely 207.38 mg/dL vs 106. 4 mg/100 ml (Faye *et al*., 2014).

The difference in blood cholesterol levels from the results of this study with other studies may be due to the feed factors used, the physiological status of the research livestock, and other environmental factors. Feeding cows with different levels of concentrate containing *C.odorata* silage resulted in no significant differences (P>0.05) in cholesterol levels in the blood. These results may indicate that the animal had sufficient nutrition. In this study, cows were provided with the same amount of concentrate and rice straw as the basal diet but with different levels of *C.odorata*. Guyton and Hall (1997) reported that insulin secretion would be inhibited in low blood glucose levels, and blood cholesterol concentrations would increase. Mitruka *et al*., (1977) indicated that the average bovine blood cholesterol level is between 80-170 mg/dl. Thus, the blood cholesterol levels of the Bali cattle in the study were above the normal range, namely 194.74 mg/dL to 220.49 mg/dL.

Table 2. Effect of treatment on Total Cholesterol, Blood Glucose, Blood Urea, Total Blood Plasma Protein, Blood Plasma Hb, Hematocrit, Erythrocytes and Leukocytes in Animal Research.

Variable	Treatment			SEM	P-value
	RCO_{30}	RCO_{40}	RCO_{50}		
Cholesterol (mg/dL)	195.74	220.49	205.91	17.14	0.89
Blood glucose (mg/dL)	91.64[a]	101.84[b]	61.57[a]	0.69	0.01
Blood urea (mg/dL)	48.41	49.04	49.44	1.30	0.82
Total blood plasma protein (g/dL)	6.30	6.93	6.08	0.20	0.66
Hb blood plasma (g/dL)	18.18	17.68	18.92	1.50	0.36
Hematocrit (%)	54.54	53.03	45.56	1.78	0.27
Erythrocytes (10^6/µl)	8.14	8.24	7.06	0.25	0.21
Leukocytes (10^3/µl)	8.84	9.62	8.56	0.31	0.26

Effect of treatment on Blood Glucose level

Blood glucose levels in this study ranged between 61.57-101.84 mg/dL with an average of 85.02 mg/dL and still in normal glucose levels. According to Mitruka *et al*. (1977), cows' normal range of blood glucose levels varies between 43-100 mg/dL. Typically, the blood glucose levels in this study are not only due to the energy factor supplied from the feed consumed by livestock but also by the control mechanism of insulin and glucagon hormones which functions to regulate the balance of blood glucose levels in the body of Bali cattle.

The average blood glucose levels found in this study were higher when compared with the results of Bira (2016) when Bali cows were fed concentrates containing *C. odorata* (85.02 mg/dL. vs 82.47 mg/dL). Whereas when compared to the results of the study by Tahuk *et al*., (2017) using forage on Bali cattle with different lengths of time of administration to Bali cattle, the results were higher (85.02 mg/dL vs 58.62 mg/dL) and when compared with the results of research conducted by Windi *et al*., (2016) which used a concentrate containing corn cobs in Bali cattle, the results were also higher, namely (85.02 mg/dL vs 64, 23 mg/dL). The difference in blood glucose levels from the results of this study with other reports from other studies may be due to the feed factors used, the physiological status of the research livestock, and other environmental factors.

Blood glucose is an indicator of food substances, especially animal protein and energy. In ruminants, glucose is needed in a certain amount for basic living needs, tissue growth, and milk production. Glucose levels in the body are controlled through glycolysis, glycogenesis, and gluconeogenesis so that the glucose concentration in the body is maintained relatively constant. Glucose is absorbed from the digestive tract in small amounts, and its levels in the blood are maintained through the synthesis of endogenous for the essential functions of body tissues. The average blood glucose obtained in this study varied between 61.57- 101.84 mg/dL, with the highest level in the treatment RCO_{40}, where cows offered 40% of *C. odorata*-based concentrates, i.e. 101.84 mg/dL, followed by RCO_{30} (40% of *C. odorata*; 91.64 mg/dL) and the lowest by RCO_{50} (61.57 mg/dL) when the animals were fed 40% of *C. odorata*-based concentrates.

The analysis of variance showed that the cows fed *C. odorata*-based concentrates had significantly (P<0.05) affected the blood glucose levels of Bali cattle. Blood glucose concentrations were significantly different between feed treatments because blood glucose was maintained by a homeostatic mechanism (Windi *et al*., 2016). Giving levels of 30%, 40%, and 50% of *C. odorata* flour in a concentrate ration mixture can have a significant effect on the degradation of feed by microbes and the metabolism of rumen microbial cells to produce propionic acid, which is a precursor for the formation of glucose in the body, causing blood glucose in the body of Bali cattle to have a significant impact. The overall effect of the treatments on the measured parameters can be seen in Figure 1.

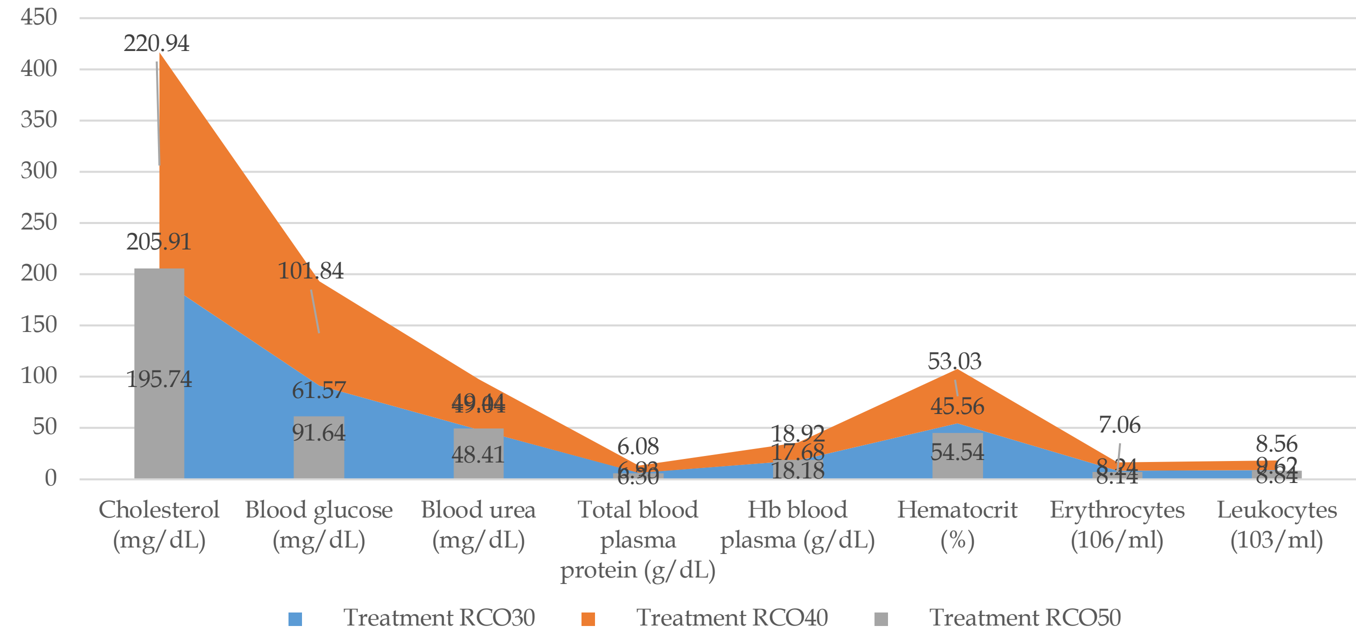

Figure 1. Effect of treatment on measured variables

Effect of treatment on Blood Urea concentration

Blood urea levels reflect Bali cattle's physiological status in response to feed consumption, especially protein and ammonia production. The highest average blood urea obtained in this study was in 50% *C. odorata* flour in the concentrate mixture (RCO_{50}); namely, 49.44 mg/dL, followed by treatment of the addition level of 40% flour. *C. odorata* in a concentrate ration mixture (RCO_{40}) which is 49.04 mg/dL and the lowest by treatment with the added level of 30% *C. odorata* flour in concentrate ration (RCO_{30}), which is 48.41 mg/dL. Thus, the mean value of male Bali cattle blood urea produced (Table 2.) varied between 48.41 mg/dL to 49.44 mg/dL with an average of 48.96 mg/dL. The average blood urea produced in this study when compared to the results of Bira (2016) study by giving concentrate made from white flower bush (*C. odorata*) to Bali cattle, the results were lower (48.96 mg/dL vs 52.60 mg/dL. Whereas when compared with the results of research by Tahuk *et al.*, (2017) using forage on Bali cattle with different lengths of time of administration to Bali cattle, the results were higher (48.96 mg/dL vs 13 mg/dL), and when compared with the results of research conducted by Windi *et al.*, (2016) which used a concentrate containing corn cobs in Bali cattle, the results were higher (48.96 mg/dL vs 27.92). mg/dL). Furthermore, if the results of this study were compared with those reported by Munzaronah *et al.*, (2010) by providing feed with different protein levels to Javanese cattle, the result was higher 48.96 mg/dL vs 44.72 mg/dL. The difference in blood urea levels from this study with the research reports conducted by the researchers may be due to the feed factors used, the physiological status of the research livestock, and other environmental factors.

The average blood urea levels produced in this study, namely 48.96 mg/dL, were still in the range of normal urea levels. According to Hungate, (2013), the normal range of blood urea levels in cattle is between 26.60 and 56.70 mg/dL. Usually, the blood urea levels produced in this study are influenced by the consumption of food substances from Bali cattle. This result is related to the study by Roseler *et al.*, (1993), that high blood urea concentrations cause livestock to be inefficient in utilising their energy. The high blood urea levels will require more energy to convert the high concentration of ammonia in the rumen fluid into blood ammonia which is then secreted in the form of urea in the urine (Purbowati, 2007).

The analysis of variance showed that the level of addition of 30%, 40%, and 50% *C. odorata* flour in the concentrate ration mixture had no significant effect ($P>0.05$) on the blood urea levels of Bali cattle. The same blood urea concentration between feed treatments was probably caused by the level of consumption of food substances, especially the absorption of amino acids and the absorption of ammonia by the same Bali cattle. In this condition, there may be a nitrogen balance in the rumen needed by the microbes of the rumen and the host livestock so that the blood urea levels of Bali cattle used in the study have the same effect.

Effect of treatment on Blood Plasma Protein

Plasma protein is a protein replacement source in tissues deficient in protein. Plasma proteins in the tissue will be broken down into amino acids (Guyton, 1983).

The speed of the formation of plasma protein by the liver depends on the concentration of amino acids in the blood, meaning that the plasma protein concentration decreases when the amino acid supply is not suitable. Conversely, when there is an excessive protein in the plasma, it is used to form tissue proteins. So there is a constant balance between plasma proteins, amino acids, and tissue proteins (Dja'far, 1988). The results of research by Utomo *et al*., (2017) on the characteristics of plasma protein in Bali cattle found that there were plasma protein bands for male and female calves (0-1.5 years old) and puberty (2-2.5 years old).), and adults (3-5 years) have protein bands of varying thickness and by Irfan *et al*., (2014) that clinical, biochemical parameters can be used to explain the mechanism of aberration and provide an overview of livestock health conditions, metabolic status, and help diagnosis so that appropriate handling of livestock can be carried out.

The highest average blood plasma protein data produced in this study was achieved by treatment with the addition of 40% *C. odorata* flour (RCO_{40}); namely, 6.90 g/dL, followed by treatment with the addition of 30% *C. odorata* flour (RCO_{30}) was 6.30 g/dL, and the lowest by treatment with the addition of 50% C. odorata flour (RCO_{50}) was 6.08 g/dL. Thus, the mean total blood plasma protein values of male Bali cattle produced (Table 2) varied from 6.08 g/dL to 6.93 g/dL with an average of 6.44 g/dL. The variance results showed that the treatment had no significant effect (P>0.05) on the total blood plasma protein.

The mean total blood plasma protein levels achieved in this study were still within the average level. According to Radostits *et al.*, (2007), the normal range of total plasma protein values in cattle is 5.7 - 8.1 g/dL. Usually the total blood plasma protein levels produced in this study were influenced by the adequate needs for food substances by livestock and the healthy condition of the livestock at the time of the study, although *C.odorata* had secondary metabolic compounds that did not interfere with the health of the animals studied. Kaslow, (2010) stated that the total plasma protein increases due to chronic infection, hypofunction of the adrenal glands, liver failure, collagen disease in blood vessels, hypersensitivity (allergies), dehydration, and respiratory disease (shortness of breath), hemolysis and leukaemia. Meanwhile, total plasma protein decreased due to malnutrition and malabsorption, liver disease, chronic and non-chronic diarrhoea, hormonal imbalance, kidney disease (proteinuria), low albumin, and low globulin.

This study's mean total blood plasma protein was higher when compared with the results of Radostits *et al*., (2007), which is between 6.44 g/dL vs 6.9 g/dL. This result was probably due to the factors such as feed used, pathological conditions, the physiological status of the animals, and other environmental factors.

Effect of the treatment on Blood Hemoglobin

The concentration of blood haemoglobin is measured based on the intensity of its colour using a photometer and is expressed in g/100 mL or g/dL. Haemoglobin levels are influenced by the adequacy of feed, especially protein and digestibility in rations, age, sex, and type of livestock (Schalm *et al*., 1975). Haemoglobin is a solid substance in the blood that causes a red colour and a protein molecule in red blood cells that functions to transport CO_2 from tissues, take oxygen from the lungs, maintain acid-base balance, and is a source of bilirubin in livestock. The presence of haemoglobin in the blood allows the ability to transport oxygen and the appearance of red colour in the blood (Frandson (1993). The highest average blood haemoglobin obtained in this study was achieved by the treatment of the addition level of 50% *C. odorata* flour (RCO_{50}; 18.92 g/dL), followed by treatment of 30% (RCO_{30} : 13.43 g/dL) and the lowest by treatment with the addition of 40% *C. odorata* (RCO_{40} :17.68 g/dL). Thus, the mean blood haemoglobin value of male Bali cattle produced (Table 2) varied between 17.68 g/dL to 18.92 g/dL with an average of 18.26 g/dL. The average blood haemoglobin level produced was still above normal levels. Coles (1980) reported that the normal range of blood haemoglobin levels in Bali cattle is 5.70 - 8.50 g/dL. Typically, the blood haemoglobin levels produced in this study are influenced by the consumption and digestibility of sufficient protein for the needs of the Bali cattle used.

The results of this study were higher when compared with that the results reported by Bira (2016) when Bali cattle offered *C. odorata*-based concentrates, which were between 18.26 g/dL vs 11.42 g/dL. Similarly, the results of this study were higher when compared with the results of Dewi *et al.* (2018) when weaned Bali cattle fed different proteins and energy (18.26 g/dL vs 10.78 g/dL). In addition, blood haemoglobin in this study was higher compared with the results of Windi *et al*., (2016) when Bali cattle offered concentrate containing corn cobs (18.26 mg/dL vs 13.56 g/dL). Furthermore, this study's results are still higher than Coles (1980) in cattle, namely (18.26 g/dL vs 7.10 g/dL. The difference in blood haemoglobin levels from this study with the research reports conducted by the researchers may be due to the feed factors used, the physiological status of the research livestock, and other environmental factors. There were no significant differences between treatments on (P>0.05) the blood haemoglobin concentration of Bali cattle. The same level of protein consumption and absorption of amino acids by Bali cattle probably caused this.

Effect of treatment on Blood Hematocrit

Hematocrit or packed cell volume (PCV) is the ratio between erythrocytes and blood plasma expressed in per cent volume. A deficiency of amino acids in the feed can cause a decrease in the percentage of hematocrit. In contrast, an increase in hematocrit is caused by dehydration, so the ratio of erythrocytes to blood plasma is above standard. A dehydrated state of the body can cause an increase in the hematocrit value, while a feed with insufficient nutrition causes reduced blood formation and decreased hematocrit value (Frandson, (1993).

Hematocrit is one of the blood parameters that reflects the proportion of cells and fluids in the blood, and usually, the level is three times the blood haemoglobin. Low levels of hematocrit can be caused by several factors, including deficiency of red blood cells (anaemia), bleeding occurs, leukaemia, destruction of red blood cells, deficiency of nutrients, excessive water consumption, iron deficiency, folic acid, vitamins B12 and B6, and damage to the spine. According to Schalm *et a*l., (1975), low hematocrit can indicate several abnormalities, including anaemia, haemorrhage, bone marrow damage, red blood cell damage, malnutrition, myeloma, rheumatoid, and arthritis. On the other hand, if the hematocrit value is high, it indicates dehydration, erythrocytosis, and venous polycythemia. Per cent blood volume (PCV) varies from species to species. Hematocrit values in mammals range from 35-45%.

This study's highest average hematocrit data was achieved by treatment with 30% C.odorata flour in the concentrate ration mixture (RCO_{30}), namely 54.54%, followed by treatment with the added level of 40% C. odorata flour. In a mixture of concentrate ration (RCO_{40}) and the lowest level by treatment with the addition of *C. odorata* flour 50% in concentrate ration mixture (RCO_{50}), that is 45.56%. Thus, the average hematocrit value of male Bali cattle produced (Table 2) varied between 45.56 to 54.54, averaging 51.04%.

The average hematocrit levels in this study were 51.04%, still within the range of normal hematocrit levels for cattle. According to Mangkoewidjojo & Smith, (1988), the normal range of hematocrit levels in Bali cattle is between 33-47%, while the hematocrit levels reported by Dharmawan (2002) are between 24 and 46%. Usually, the levels of hematocrit produced in this study are influenced by the fulfilment of the needs of food substances from research livestock (especially protein, amino acids, iron, minerals, and vitamins); average blood erythrocyte level; no anaemia occurs by livestock; There were no animal health problems during the study, even though *C. odorata* contains secondary metabolic compounds, which, if consumed too much, cause health problems in livestock. However, giving up to the level of 50% has not given a negative response to livestock.

The analysis of variance showed that the level of addition of *C. odorata* 30%, 40%, and 50% in the concentrate ration mixture had no significant effect ($P>0.05$) on the hematocrit levels of Bali cattle. The level of consumption of substances and absorption of amino acids by the same Bali cattle probably caused the same hematocrit concentration between feed treatments. The average hematocrit levels produced in this study when compared with the results of Dewi *et al*., (2018) using feed with different protein and energy content in weaning Bali cows, the results were higher (51.04 vs 33.15 %), and when compared with the results of research conducted by Roland *et al*., (2014) on cattle, the results are much higher (51.04 vs 26%). The difference in the levels of hematocrit from this study with the research reports conducted by the researchers may be due to the factor of feed used, the physiological status of the research livestock, and other environmental factors.

Effect of treatment on Blood Erythrocyte

The primary function of erythrocytes is to transport haemoglobin and oxygen from the lungs to all body tissues. Cattle are considered anemic if their erythrocyte count is below the usual range. Erythrocytes consist of 65% water and 33% Hb; the rest are fat stoma cells, minerals, vitamins, other organic materials, and K ions (Oematan *et al*., 2023). This result is due to inadequate nutrition, so that blood formation is not good (Utama *et al*., 2001).

The highest erythrocyte average data produced in this study was achieved by the treatment of the addition level of 40% C. odorata flour in a concentrate ration mixture (RCO_{40}), namely 8.24 x $10^6/\mu L$ followed by a treatment level of addition of 30% flour. C. odorata in a concentrate ration mixture (RCO_{30}) which is 8.14 x $10^6/\mu L$ and the lowest by treatment with the added level of C. odorata 50% in the concentrate ration mixture (RCO_{50}), which is 7.06 x $10^6/\mu L$. Thus, the mean value of male Bali cattle erythrocytes (Table 2) varied between 7.06 x $10^6/\mu L$ to 8.24 x $10^6/\mu L$ with an average of 7.81 x $10^6/\mu L$.

The mean erythrocyte levels were 7.81 x 106/µL, still within the range of normal erythrocyte levels. According to Dharmawan (2002), Bali cattle's normal range of erythrocyte levels is between 5.0 - 10.0 x $10^6/\mu L$. Typically the erythrocyte levels produced in this study are influenced by the consumption of food substances, especially amino acids, iron, minerals, and vitamins which are good enough for the needs of erythrocyte formation, and Bali cattle used in this study do not experience anaemia. The levels of livestock erythrocytes are known to be significantly impacted by the availability of appropriate feed components to suit livestock demands (Adam *et al*., 2015). Feeding nutrients such as amino acids, iron, Vitamins, and Cu are essential components affecting livestock's erythrocyte levels Putriningsih and Arjentinia, (2015) (Frandson, 1993). Several vitamins and minerals play an essential role in the erythropoiesis process (Adam *et al*., (2015) and Dewi *et al*., (2018). Iron is required for heme synthesis. Copper in ceruloplasmin is needed to release iron from plasma tissue, while vitamin B6 acts as a cofactor in the enzymatic heme synthesis stage. Cobalt is needed in the synthesis of vitamin B12 in ruminants. The deficiency of vitamin B12 and folic acid can cause the failure of maturation in the maturation of red blood cells (erythropoiesis), which causes the total internal erythrocytes to be below. In addition to dietary factors such as amino acids, iron, vitamins, and Cu can affect erythrocyte levels, consumption of tannins over a long time can affect blood erythrocyte levels even though the content of tannins can function as antioxidants in the body (Delimont *et al*., 2017). However, the tannin content in the research ratio did not affect the erythrocyte levels of the research livestock, so it did not interfere with livestock health in the form of anaemia.

The analysis of variance showed that the application of 30%, 40%, and 50% of *C.odorata* flour in the concentrate ration mixture had no significant effect (P>0.05) on the erythrocyte levels of Bali cattle. The same level of consumption and absorption of amino acids by Bali cattle probably caused the same erythrocyte concentration between feed treatments.

The average erythrocyte yield in this study, when compared with the results of Bira (2016) study by giving a concentrate made from silage made from white flower bush (*C. odorata*) to Bali cattle, the results were lower (7.06×10^6 to $8.24 \times 10^6/\mu L$) vs 10.70×10^6 to $14.50 \times 10^6/ml$). Meanwhile, when compared with the results of research by Dewi *et al*., (2018) on Bali cows weaning off using feed with different protein and energy content, the results are almost the same, namely (7.06×10^6 to $8.24 \times 10^6/\mu L$ vs 6.60×10^6 to $8.90 \times 10^6/\mu L$) and when compared with the results of research conducted by Roland *et al*., (2014) on cattle, the results are relatively the same, namely (7.06×10^6 to $8.24 \times 10^6/\mu L$ vs 4.90×10^6 to $10.0 \times 10^6/\mu L$). The difference in erythrocyte levels from this study with the research reports conducted by the researchers may be due to the feed factors used, the physiological status of the research livestock, and other environmental factors.

Effect of treatment on Leukocyte

Leukocytes can indicate deviation in organ function or infection of infectious agents and foreign bodies and support clinical diagnosis in animals (Frandson, 1993). In general, total and differential leukocytes can provide an overview of and health status of animals (Sugiharto, 2016). Guyton and Hall (1997) stated that total leukocytes reflect the level of health influenced by several internal factors, including gender, age, disease, and hormones, as well as external factors, such as environmental conditions, livestock activity, stress, and feed. Furthermore, Isroli *et al*., (2009) state that to determine the level of immunity, it can be seen from blood variables in the form of leukocytes and a total differential of leukocytes.

The highest average leucocyte data produced in this study was achieved by the treatment of the level of adding 40% *C. odorata* flour in a concentrated mixture (RCO_{40}), namely $9.62 \times 10^3/\mu L$. They are following adding 30% flour *C. odorata* in a concentrate ration (RCO_{30}), which is $8.84 \times 10^3/\mu L$ and the lowest by treatment with the addition of 50% C. odorata flour in a concentrate ration mixture (RCO_{50}), which is $8.56 \times 10^3/\mu L$. Thus, the average leucocyte values of male Bali cattle produced (Table 2) varied between $8.56 \times 10^3/\mu L$ to $9.62 \times 10^3/\mu L$ with an average of $9.01 \times 10^3/\mu L$.

The average level of leukocytes: $9.01 \times 10^3/\mu L$, was still in the range of normal leukocyte levels. According to Dharmawan (2002) that the total average leukocyte value for Bali cattle is $4 \times 10^3/\mu L$ - $12 \times 10^3/\mu L$, while according to Brooks *et al*., (2022), it is 5.1 - $13.3 \times 10^3/\mu L$. Typically, the levels of leukocytes produced in this study were influenced by feed and healthy animal health during the study. However, it is known that *C. odorata* contains secondary metabolic compounds; the administration level of up to 50% has not been given a negative response to livestock health. The Bali cattle infected with dermatosis have high total leukocytes and monocytes compared to ordinary Bali cows (Nurani et al., 2017).

The analysis of variance showed that the level of *C. odorata* addition of 30%, 40% and 50% in the concentrate ration mixture had no significant effect (P>0.05) on the blood leukocyte levels of the research cattle. The same leucocyte concentration between feed treatments was more due to the composition and content of food substances used by the same Bali cattle. The function of leukocytes is to protect the body from pathogens using phagocytosis, producing antibodies and factors that determine the number of leukocytes, including biological activity, environmental conditions, age, and feed (Hartoyo *et al*., 2018).

The average leucocyte produced in this study, when compared with the results of the study by Pawitri *et al*., (2014) in Bali cattle, results were not much different, namely (9.01 x 103/μL vs 8.3 x 103/μL). Meanwhile, when compared with the results of research by Nurani *et al.,* (2017), the results were higher, namely (9.01 x 103/μL vs 6.98 x 103/μL. The difference in leukocyte levels from this study with the results of research reports conducted by other researchers may be due to the feed used, the physiological status of the research livestock, and other environmental factors.

Conclusion

The results showed that the administration of *C.odorata*-based concentrate to Bali cattle with different levels (30%, 40%, and 50%) had an effect on blood glucose but not on other blood variables. Therefore, *C.odorata* can be used in concentrates to increase the productivity of Bali cattle.

Author Contributions

Each author contributes in some way to the completion of this research activity. The main author provides basic ideas and provides research materials and the second, third, fourth authors design research methods and furthermore, all authors share responsibility for data collection, data tabulation and analysis, review process, and article writing.

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Conflicts of Interest

Regarding this study, the author declares that there is no conflict of interest.

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