

EFFECT OF HUMIC ACID AND MIXED HERBS ON PHYSIOLOGICAL, HEMATOLOGICAL AND BIOCHEMICAL VALUES ON GOATS IN INDONESIA**Dwatmadji,
Prof.****T. Suteky
Associate Prof.****E. Soetrisno
Associate Prof.****Dept. Animal Science Faculty of Agriculture University of Bengkulu-Indonesia****E-mail: dwatmadji.2008@gmail.com****ABSTRACT**

This study was aimed to determine the effect of mixed herbs and humic acid supplementation on the physiological status, haematological, and biochemical values of local goats in Indonesia. Twenty-four local goats 7-8 months old, were divided into four groups of six goats each. T0: herbs without humic acid, T1: herbs with 2-gram humic acid/goat T2: herbs and 4-gram humic acid/goat; T3: herbs and 6-gram humic acid/goat. The goats were fed grass and fermented oil palm. The result found that Orthogonal contrasts detect ($P < 0.001$) a significant influence of humic acid addition on respiration and heart rate, as well as HTC, with the amount of addition varying considerably during the 12.00 observation. There are no alterations in erythrocyte indices, leucocytes increase quantitatively with neutrophils rising significantly in line with the presence of humic acid. Humic acid lowers serum cholesterol while boosting protein levels in the blood. In summary, humic acid impacts physiological and haemato-biochemical parameter of local goats in Indonesia.

Keywords: Curcuma, HTC, cholesterol, total protein

وآخرون

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تأثير حامض الهيوميك ومجموعة من الاعشاب في الصفات الفسلجية والدمية والقيم الكيموحيوية في الماعز في إندونيسيا

سوترينو
استاذ مشاركسوتيكي
استاذ مشاركدوات ماجي
استاذ**المستخلص**

هدفت هذه الدراسة إلى تحديد تأثير مكملات مجموعة من الأعشاب وحامض الهيوميك على الحالة الفسيولوجية والقيم الدموية والكيميائية الحيوية للماعز المحلية في إندونيسيا. تم تقسيم أربعة وعشرين من الماعز المحلية التي تتراوح أعمارها بين 7-8 أشهر إلى أربع مجموعات تضم كل منها ستة من ماعز: T0: أعشاب بدون حامض الهيوميك، T1: أعشاب تحتوي على 2 غم من حامض الهيوميك/ماعز، T2: أعشاب و 4 غم من حامض الهيوميك / ماعز؛ T3: أعشاب و 6 غم من حامض الهيوميك / ماعز. تم تغذية الماعز على العشب ونخيل الزيت المخمر. أظهرت النتائج التباينات المتعامدة عن وجود معنوية ($P < 0.001$) كتأثير كبير لإضافة حامض الهيوميك في التنفس ومعدل ضربات القلب، وكذلك HTC، مع اختلاف كمية الإضافة بشكل كبير أثناء الملاحظة 12.00. لا توجد تغييرات في مؤشرات كريات الدم الحمراء، تزداد كريات الدم البيضاء كمياً مع ارتفاع حامض الهيوميك. يعمل حامض الهيوميك على خفض نسبة الكوليسترول في الدم مع تعزيز مستويات البروتين. باختصار، يؤثر حامض الهيوميك في المعايير الفسيولوجية والكيميائية الحيوية للدم لدى الماعز المحلية في إندونيسيا.

الكلمات المفتاحية: الكركم، HTC، كوليسترول، البروتين الكلي

INTRODUCTION

The greatest substitute source to deal with the year-round lack of grass is to use agricultural waste as goat feed. (16, 27) However, that waste cannot provide the nutrients needed by goats for optimal performance. Herbal supplementation has the potential to increase the absorption of nutrition and enhance performance (21). Suteky et al. (41) found that polyherbal supplementation increases average daily gain in Peranakan Ettawa goats. Humic acid is another natural supplement that may be advantageous to the composition of microorganisms in ruminants' digestive systems (22). Humic acid is already used commercially abroad to improve feed efficiency in dairy goats, cattle, pigs and poultry. Various research showed inconsistent results, the provision of humic acid has no effect on blood parameters (13) decreases cholesterol (22), and increases blood protein (10). Sizmaz *et al.* (38) reported that the addition of humic and boric acid tends to increase rumen fermentation parameters such as butyric acid, propionate, acetate, valerate concentration as well as total VFA. The biochemical and haematological properties of blood are commonly used to utilize and assess the health physiological status and nutritional state of livestock (11, 18, 19, 39). Blood composition analysis has been used as a marker to measure the effectiveness of dietary nutrients and supplements (29). Yadav et al. (43) also noticed that goat immunity can also be evaluated using biochemical and haematological profiles. Analysis of blood constituents may be widely employed as a marker to evaluate the effectiveness of feed supplements and nutrient content. Abdulkareem (1) found that blood alterations can be used to detect nutritional deficiencies and anomalies in the metabolism of riverine buffaloes during the calving and postpartum periods. The blood system is a crucial indicator of a biological response to stress and is temperature-sensitive. Numerous factors, including breed, species, age, nutrition, sex diseases, physiological conditions, and seasonal fluctuations, can alter the haematological findings (19 and 25). Interpreting the results of a haematological and biochemical analysis, it is important to

consider the animal's breed, sex, nutrition, age, physiological state, management as well as climate (31). The aim of this investigation was to assess the impact of humic acid and mixed herbs on local goats' haemato-biochemical value and physiological response.

MATERIALS AND METHODS

Study site: The study was carried out in the University of Bengkulu's Animal Science Laboratory, an experimental farm in Seluma, Bengkulu, approximately 23 kilometres from Bengkulu University (located at -35.9018300 latitude and 102.360021 longitudes).

Experimental animals: In this research, local does were used as experimental animals. Twenty-four does aged 10-12 months with a BW of 22.35 ± 3.99 , were divided into four treatments, each with six does. The treatments were T0: mix-herbs without humic acid T1: mix-herbs with 2-gram humic acid/doe T2: mix-herbs and 4-gram humic acid per doe; T3: mix-herbs and 6-gram humic acid per doe. All does were kept separately in 140 x 80-centimetre cages, and fed with fermented oil palm sludge, forage, rice bran, and tofu waste. This fed formulation has a CP of 18% and a GE of 3438 Kal/g. A 5 L plastic bucket containing water was made available to all goats.

Mixed herbs preparation

All herbs (*Curcuma manga*, *Andrographis paniculata*, *Melastoma malabathricum* and *Nigella sativa*) in powder form purchased from local pharmacies were used in this experiment. The herbs mixed with other ingredients (Table 1) are then fed into the feed pellet machine.

Table 1. Mixed herb supplementation

Nutrient composition (fresh weight)	Weight (g)
Rice bran	300
Concentrate	100
Molasses	250
Cassava flour	100
Mineral	10
Salt	40
<i>Curcuma mangga</i>	50
<i>Andrographis paniculate</i>	50
<i>Melastoma malabathricum</i>	50
<i>Nigella sativa</i>	50
Total	1000

Herbs supplementation (50 grams) and humic acid are given in the morning at 08:00, followed by tofu waste (2%), forage (4%) and oil palm sludge ad libitum.

Parameter measured

Three measurements of the temperature (°C) and relative humidity (%) were made each day at 8:00, 12:00, and 16:00. At the same time the thermoregulation analysis was done based on the given physiological responses which consisted of the rectal temperature, pulse rate and respiratory rate. Thermal Humidity Index/ THI = $(1,8 \times T + 32) - [(0,55 - 0,0055 \times RH) \times (1,8 \times T - 26)]$ after Thompson dan Dahl (42). While the Heat tolerance coefficient is based on Benezra $HTC = (RT/39,44) + (RR/24)$. Three ml of whole blood was drawn from each animal jugular vein before feeding at 8 a.m. Blood collection was performed based on the Animal Care University of Bengkulu. The Automated Hematology Analyzer Mind ray 2600 was used to measure blood parameters such as RBC (Red Blood Cell), and HCT/PCV (Packed Cell Volume), haemoglobin, as well as WBC (White Blood Cell),). While MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), and MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated based on RBC, Hb, and PCV. The remaining 1.5 ml of blood was placed into non-anticoagulant sterile serum tubes to assess glucose, total

cholesterol, total protein, albumin, and globulin levels (The CE IVD Dialab).

Statistical Analysis: All data were analysed using ANOVA (IBM SPSS V 24.) to compare treatment means of control versus supplemented groups and humic acid addition were evaluated through orthogonal contrast (T0 vs T1-3); (T1 vs T2 + T3) and (T2 vs T1-3); (T1 vs T2 + T3) and (T2 vs T3). Comparison among ambient temperature, humidity and THI was done using the Duncan Multiple Range Test (12).

RESULTS AND DISCUSSION

The results revealed that the temperature during the experimental period was 24.2-30.8°C. The temperature at 08:00 was significantly lower ($P < 0.001$) compared to the temperature at noon and afternoon observation. The highest THI was found at 12.00 of 83.4. According to data from the Meteorological, Climatological, and Geophysical Agency, the surrounding temperature and humidity during the trial were approximately 17-33 Celsius and 60-100%, respectively. The ideal ambient temperature of goats varies depending on breed, gender, nutritional and physiological health (23). According to Ribeiro et al. (31) Anglo Nubian goats can adapt to hot temperatures up to 36°C, and even South American goats (Azul) could adjust to extreme conditions of 40°C.

Table 2. Environmental conditions in Bengkulu Province Indonesia during the trial

Variables	Time of Measurement					
	08:00	12:00	16:00	Ave	SEM	P value
Temperature (C)	24.2 ^a	30.8 ^c	28.0 ^b	27.7	0.14	0.001
Humidity (%)	91.6 ^c	75.2 ^a	85.6 ^b	84.1	0.37	0.001
Thermal Humidity Index	74.8 ^a	83.4 ^c	80.5 ^b	79.5	0.19	0.001

Means with different superscripts within each column differ significantly ($P \leq 0.001$)

THI was utilized to show the possible threat to livestock as a result of environmental change. Our findings showed that THI was found to be 74.8-83.4, THI indices are classified to show the degree of heat stress (8). THI less than 80 was considered a normal environment for goats, alert between THI 80 and 85, dangerous between 85 and 90, and extreme when THI over 90 (37). This classification differs from

the classification in dairy cows which THI values less than 72 indicate that cows are not stressed, 72 to 78 demonstrate that cows are stressed, and over 78 shows that cows were incapable of preserving thermoregulatory components or normal body temperature (Agricultural Engineering Technology Guide, Clemson University, Clemson, Sc. 29634, USA).

Table 3. Physiological value of goats supplementing with herbs and humic acids

Variables	Treatments				P value		
	T0	T1	T2	T3	T0 vs T1-3	T1 vs T2-3	T2 vs T3
08:00							
Respiration rate (x/min)	24.63	24.57	24.5	24.53	0.713	0.862	0.92
Pulse rate (x/min)	80.00	79.63	79.23	79.63	0.571	0.831	0.711
Rectal temp (oC)	38.67	38.7	38.66	38.69	0.407	0.328	0.31
HTC	2.01	2.00	2.00	2.00	0.673	0.881	0.906
12:00							
Respiration rate (x/min)	26.57	25.37	25.87	26.1	**	**	ns
Pulse rate (x/min)	85.17	83.23	82.9	83.4	**	ns	ns
Rectal temp (oC)	38.84	38.8	38.81	38.83	ns	ns	ns
HTC	2.43	2.38	2.4	2.41	**	ns	ns
16:00							
Respiration rate (x/min)	23.43	24.17	24.2	24.23	**	ns	ns
Pulse rate (x/min)	85.17	83.23	82.9	83.4	ns	ns	ns
Rectal temp (oC)	38.65	38.69	38.67	38.67	ns	ns	ns
HTC	1.96	1.99	1.99	1.99	**	ns	ns

HTC: Heat Tolerance Coefficient; NS: Non –Significant

Morning measurements revealed that the addition of herbs and humic acid did not affect respiration rate, pulse rate, rectal temperature, and HTC. The effect of ambient temperature was discovered to raise the respiration rate per minute, this finding is in agreement with Sarangi (33). During the noon examinations, orthogonal contrasts detect ($P<0.001$) a highly significant influence of humic acid addition on respiration and pulse rate as well as HTC (T0 vs T1-T3) and the effect was considerably dependent on quantity of humic acid added. Respiration rate is an effective and reliable marker of heat stress (35). Our findings show that respiration rate in normal conditions. According to Sejian et al (35) under

thermoneutral conditions, a goat's respiratory rate ranges from 15 to 30 breaths per minute. Based on afternoon observations, humic acid treatment did not influence pulse rate or rectal temperature. Goats are homeothermic creatures, which implies they can keep a generally consistent body temperature (23). It can be seen in Table HTC goats treated with humic acid performed better which indicates those HTC were closer to normal than the control. Heat Tolerance Coefficient (HTC) can be used to detect an individual animal's adaptability levels in reaction to diverse environmental circumstances and the ideal values for HTC were 2/two (4). Increasing HTC value at noon is correlated with THI.

Table 4. Erythrocyte indices on blood goat acids of goat supplementing with herbs and humic acids

Erythrocyte indices	Treatments				Contras		
	T0	T1	T2	T3	T0 vs T1-3	T1 vs T2-3	T2 vs T3
Hb (g/dL)	9.20	9.72	9.80	9.67	ns	ns	ns
PCV (%)	22.43	25.53	23.88	25.10	ns	ns	ns
RBC ($10^6/\mu\text{L}$)	2.89	3.38	2.64	2.91	ns	ns	ns
MCV	79.66	82.16	90.93	86.12	ns	ns	ns
MCH	32.76	31.31	37.35	33.19	ns	ns	ns
MCHC	41.15	38.14	41.09	38.63	ns	ns	ns

Table 4 showed that Haemoglobin (g/dL), PCV (%), RBC ($10^6/\mu\text{L}$) MCH, MCV, and MCHC levels were unaffected by the

treatment. In line with our findings, Ikyume et al. (17) reported that supplementing with different amounts of garlic powder had no

effect on haematological indices Haemoglobin concentration in this study was 9.2-9.80 g/dL this value is in a normal range according to Bamerny et al. (7), Mohammed et al. (26) and Soul et al. (39), the value of haemoglobin is heavily influenced by age, breed, nutrition, and diseases. The percentage of PCV in this research was 22.43-25.53 which means that goats are in healthy condition. The value of goats' PCV according to several researchers was 21.29-22.8 (19) 28.6-52.0 (3), the PCV value (23.2-30.6%) depending on the goats' breed (31). RBC observed in this research was very low ($2.89-3.38(10^6/u)$) compared to other findings by Alam et al. (3) 8.3-13.06. Generally, the normal value of RBC in goats based on Laboratory Reference Values: Haematology was 8-18 ($\times 10^6/uL$), while our findings were 2.64-3.38. It does not imply that goats are in an anaemic condition because almost the same results were published by Sarmin et al. (34) RBC value of some Indonesian goats such as Ettawa Crossbred is 1.8-3.79 ($10^6/\mu L$) and Sapera goats 1.71-3.72 ($10^6/\mu L$). RBC levels ranged from 2.19 to 2.58

in a study by Ajagbe et al. (2) on West African dwarf. In addition, Babale et al. (5) also reported RBC in Sokoto Red Goats feed with different levels of Maize bran was 3.85-6.5 ($10^6/\mu L$). Additionally, the findings discovered that supplementing with humic acid and mixed herbs did not affect RBC. According to Hadi and Al-Saadi (14), there was no discernible effect on the RBC of sheep supplemented with *Rhus Coriaria* and Fibrolytic enzymes or their combination, the noticeable effect of increased RBC was observed after 3 months of administration. Differences in values discovered in this study and previous publications could be cause by several factors such as nutrition, breed, management climate etc. The result of MCV is relatively high (79-90) due to a low level of RBC. MCV value of 52-64.25 was reported by Egbe-Nwiyi et al. (9) and Soul et al. (39). The results demonstrate that humic acid supplementation has no influence on MCH and MCHC, which have values of 31.31-37.35 and 38.14-41.15, respectively.

Table 5. White blood cell and differential leucocyte counts of goat supplementing with herbs and humic acids

	Treatments				Contrasts		
	T0	T1	T2	T3	T0 vs T1-3	T1 vs T2-3	T2 vs T3
WBC ($\times/\mu L$)	9512	13050	13250	14050	ns	ns	ns
Neutrophil seg (%)	46.25	60.75	66.5	71.25	**	**	ns
Neutrophil seg ($\times 10^3/mL$)	4.57	7.98	8.84	10.02	*	ns	ns
Neutrophil band (%)	4.25	2.5	1.75	2	**	ns	ns
Neutrophil band	389	315	238	281	**	ns	ns
Lymphocytes (%)	44.5	32.5	29.5	23.75	**	ns	ns
Lymphocytes ($\times 10^3/mL$)	4.05	4.20	3.88	3.32	ns	ns	ns
NLR	1.23	2	2.37	3.09	**	*	*
Monosit	5	4.25	2.25	3	*	*	ns
Basofil	0.00	0.00	0.00	0.00	ns	ns	ns
Eosinophil	0.00	0.00	0.00	0.25	ns	ns	ns

NLR: Neutrophil Lymphocytes Ratio

The result showed non significant differences in WBC, however, it seems that the addition of humic acid increased WBC numerically. As the function of WBC is a part of the immune system (32), humic acid supplementation appears to have a protective system that provides a rapid and robust defence against any pathogenic agent, which is most likely

related to supplementation. Unlike other ruminants, goats have a higher concentration of lymphocytes in their blood than neutrophils; nevertheless, our findings are the opposite. Our results are consistent with Shaikat et al. (36) the percentages of neutrophils 77.64 (51.44–99) and lymphocytes 17.21 (1.6-38) on also in Barbari goats' neutrophils 56.8% while lymphocytes 36.35% (25). Humic acid

supplementation may lead to a higher percentage of neutrophils. The addition of 100g of HA to a cow's ration each day improved nutrition uptake by boosting the activity of neutrophil, which could protect against pathogenic bacterial and lowering mortality from severe bacterial infections. (15). The addition of humic acid/goat raises blood glucose levels with the level of 4 and 6 grams/goat (Table 6). This finding is consistent with the findings of Hassan et al. (15). According to Hassan et al. (15), humic acid added to dairy cows caused a linear rise ($P<0.05$) blood glucose and a decrease in

cholesterol. Serum cholesterol decreases significantly with the addition of 4 grams of humic acid. Serum cholesterol concentration varies greatly depending on several factors. Maythem et al. (24) reported cholesterol concentration Awassi Ewes 73.63-110.27 mg/dL depending on the stage of pregnancy. Moreover, Albulkareem (1) noted cholesterol concentrations (141.81- 151.53 mg/dl) did not change during the calving and postpartum period on Iraqi riverine buffaloes. While Bamerny et al. (7) found cholesterol of 54.2-82.2 mg/dL in local Black goats.

Table 6. Biochemical value of goat supplementing with herbs and humic acids

Variables	Treatments				Contrasts		
	T0	T1	T2	T3	T0 vs T1-3	T1 vs T2-3	T2 vs T3
Glucose mg/dl	31.5	25.0	34.3	37.5	ns	**	ns
Cholesterol	154.0	153.5	127.3	154.5	ns	ns	*
Total protein mg/dl	4.4	5.1	4.9	5.0	**	ns	ns
Albumin mg/dl	3.4	3.7	3.4	3.6	ns	ns	ns
Globulin mg/dl	1.2	1.4	1.5	1.4	ns	ns	ns
Ratio A/G	3.0	2.6	2.4	2.7	ns	ns	ns

Ration A/G: Ratio Albumin/Globulin

Blood proteins can be used to assess metabolic activity and intestinal health. Blood protein increased significantly in the group of goats with herbs and humic acid supplementations and no significant differences among the levels of humic acid. Sultan (40) found that the addition of *Curcuma sp* increases total protein. Serum albumin in this study was almost similar to the findings by Bairwa et al. 3.55 g/dL (6), 2.39-3.35 (7); 2.7-3.7 (16) g/dL; 2.39-2.6 (27). Results also revealed that the levels of serum albumin, globulin, and Ratio A/G did not differ significantly across treatments, however, those levels were numerically higher than the control. The results demonstrate that orthogonal contrasts detect a substantial effect of humic acid addition on respiration, heart rate, and HTC ($P<0.001$), with the amount of addition varying significantly during the 12.00 observation. Erythrocyte indices (RBC, PCV, Hb, MCV, MCH and MCHC) do not alter, but leucocytes and neutrophils increase quantitatively in the presence of humic acid. Humic acid lowers serum cholesterol while boosting protein levels in the serum. No effect on albumin, globulin and their ratio.

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