Scholars Journal of Agriculture and Veterinary Sciences (SJAVS) **e-ISSN 2348–1854**
Abbreviated Key Title: Sch. J. Agric. Vet. Sci. **p-ISSN 2348–8883**
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Effects of the Supplementation of *Euphorbia hirta* L. in Feed on the *In-Vitro* Rumen Fermentation and Digestibility

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Original Research Article

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Article History
Received: 03.04.2017
Accepted: 14.04.2018
Published: 30.04.2018

DOI:
10.36347/sjavs.2018.v05i04.009

Abstract: The purpose of this research was to analyze the effect of Patikan Kerbau (*Euphorbia hirta* L) flour on the *in-vitro* rumen fermentation and digestibility using goat rumen fluid. The materials of the research were rumen fluid of goats, forage, tofu waste and *E. hirta* flour. The research method was experimental using Randomized Block Design with 6 treatments and 4 groups of goat rumen fluid as replication. The treatments of this experiment were herbal supplements in feed with different formulations: P_0= control feed (forage 60%: concentrate 40%); P_1= P_0 + 5% *E. hirta* (dry matter basis); P_2= P_0 + 10% *E. hirta*; P_3= P_0 + 15% *E. hirta*; P_4= P_0 + 20% *E. hirta*, and P_5= P_0 + 25% *E. hirta*. The measured variables were pH rumen fluid, concentration of N-NH_3, concentration of total VFA, dry matter digestibility and organic matter digestibility in the rumen fluid incubation 48 hour. Data were analyzed using variance (ANOVA), if there any differences in treatment, then continued with Duncan Multiple Range Test (DMRT). The result of the research showed that supplementation of *E. hirta* in feed did not significantly ($P>0.05$) affect on rumen pH, but it highly significant affected ($P<0.01$) on the total VFA concentration, N-NH_3 concentration, dry matter and organic matter digestibility of feed. Conclusion of the research was the supplementation of *E. hirta* can affect the *in-vitro* rumen fermentation and digestibility. The best treatment is the supplementation of *E. hirta* flour 15% (P3) in feed because it can increase the concentration of total VFA, concentration of N-NH_3, dry matter *in vitro* digestibility and organic matter *in vitro* digestibility without changing the rumen fluid pH.

Keywords: In-vitro, fermentation, digestibility, Euphorbia hirta, supplementation, goat rumen fluid.

INTRODUCTION

Many ways can be done to increase the milk production such as giving proper and supplementing with additive feed in the form of synthetic compounds. This would influence the increase of the substrate flow to mammary gland and also the increase of the number of cell secretion of mammary gland. However, arbitrary and long-term use of synthetic products could also provide the side effects that could reduce milk production, immunity and reproductive performance of animals. Currently, galactogogues herbs are developed to obtain the safe milk production. The use of galactogogues from herbs seems to reinforce the health of dairy cattle without causing damage on tissue reaction [1], does not have side effects and does not leave residue in tissue, secretion, excretion and milk [2]. One of medicinal plants that have properties of galactogogue is herb *E hirta*.

E hirta is a wild plant commonly found in tropical regions, and has a reputation for increasing milk flow in women [3] and when it is given to female rabbits before puberty it can improve the development of mammary glands and cause secretion [4]. The ability of *E. hirta* to increase milk production involves active compounds that have the property to increase the secretion of prolactin (the main hormone of lactation), and increase the secretion of β-casein (the main protein of milk), and as a result they increase milk secretion in women and animals [5]. The phytochemical screening of *E. hirta* indicated the presence of alkaloids, flavonoids, tannins, phenolics, steroids, saponins and glycosides, whereas terpenoids was absent [6].

Feeding *E. hirta* at a dose of 10 g/kg body weight is non-toxic because there is no apparent change in behavior, food and water intake in Swiss mice and is relatively safe when administered orally [7]. There is a positive effects of *E. hirta* supplementation in feed on the intestinal microflora, histomorphology of the small intestine and the performance in broiler chickens [8]. The use of this plant in ruminants has not been studied

yet, therefore it is necessary to test the feasibility of using in in ruminant because the process of digestion of ruminants is very specific due to the presence of microbes in the rumen. This research was intended to obtain preliminary information about the effects of supplementing *E. hirta* in feed of goats on the *in-vitro* digestibility and the rumen environment.

MATERIALS AND METHODS

Experimental site and materials

In-vitro experiment and variable analysis were conducted at the Laboratory of Nutrition and Feed Ruminants, Animal Science Faculty of Andalas University, from Augustus 2016 to October 2016. Plant *E. hirta* obtained from the area around in IV Angkat subdistrict Agam regency, West Sumatera, Indonesia.

The materials used in this research were goats rumen liquid obtained from slaughterhouse, McDougall's buffer solution, flour of whole plants (*E. hirta*), forage crops and concentrate was tofu dregs. The nutritional content of the rationing material can be seen in Table-1.

Table-1: The nutritional content of Feeds Materials (%).

Materials	Nutritional Content **(% DM)**						
	DM	**CP**	**CF**	**CF**	**Ash**	**BETN**	**TDN***
Forage	89,37	10,83	28,64	1,73	9,70	49,11	54,41
Concentrate	89,88	24,05	17,58	1,70	2,73	53,94	70,85
E. hirta	94,19	18,41	21,44	1,43	9,98	48,74	61,28

Experimental design and procedures

The design of experiment was Randomized Block Design (RBD) with 6 treatments and 4 groups of goat rumen liquid as replications. The treatments were supplementation of *E. hirta* in feed with the following diet formulations: P_0 = control feed (60% forage and 40% concentrate based on dry matter); P_1 = (P_0 + 5% *E. hirta*); P_2 = (P_0 + 10% *E. hirta*); P_3 = (P_0 + 15% *E. hirta*); P_4 = (P_0 + 20% *E. hirta*) and P_5 = (P_0 + 25% *E. hirta*).

The *in-vitro* method followed procedures of metode McDougall by [9]. One gram sample of feed (dry oven 60 ºC) is fed into the fermentor tube. Meanwhile, rumen fluid was filtered at 38-39 ºC. Likewise, McDougall solution was prepared. Furthermore, rumen fluid and McDougall solution with a ratio of 1: 4 were added to the feed sample, then saturated with CO2 gas for 30 seconds and covered with a ventilated rubber cap. The next stage was incubated for 48 hours at a temperature of 38-39 ºC. After that, centrifugation was done with a speed of 2,500 rpm for 15 minutes. The next step was the addition of pepsin and then incubated again at a temperature of 38-39 ºC for 48 hours. To end the fermentation process, 0.2 ml of saturated $HgCl_2$ solution was added to kill microbes and then centrifuged at 2,500 rpm for 15 minutes. Part supernatant was used for the analysis of VFA and N-NH3. Part precipitate was filtered with Whatman filter paper, dried in an oven at 105 °C for 24 hours, then put into exicator and weighed to determine the final weight of the dry matter. The precipitate was then burned in an electric furnace and weighed to determine the content of residual organic matter digestion in-vitro.

Measurement of the in-vitro rumen fermentation and digestibility

Parameters measured included ruminal pH, total VFA and N-NH_3 concentrations of rumen fluid, dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD).

Ruminal pH was standardize the pH meter by dipping the cathode present at pH meters into buffer pH 7 before use, then dipped into the supernatant, then left for a few seconds and the emerging number represents the degree of acidity of the rumen fluid (pH).

Total VFA measurement using "Steam distillation" method. Five ml of supernatant fluid were inserted into distillation tube, then 15% H_2SO_4 added 1 ml, then the tube was closed so that it was airtight and connected with coolant flask (Leibiq type). After that the tube was directly inserted into a distillers flask containing boiling water (heated during distillation). The hot water vapor urges the VFA to condense in the coolant. The formed water was accommodated in Erlemeyer containing 5 ml of NaOH 0.5 N solution up to about 300 ml. Destilat accommodated by phenolpthalein (PP) indicator 2 drops and then titrated with HCl 0.5 N until the color change from pink become colorless. Tumed titration of 5 ml NaOH was also performed. Total VFA production is calculated by the equation:

$$\text{VFA total (mM)} = (a-b) \times N\ HCl \times 1000/5.$$

N-NH3 concentration were determined using the Conway diffusion micro technique. The Conway Cup consists of three chambers. In the center there was a small cup and two other rooms were located outside the circle. The measurement step begins by inserting a 1 ml boric acid into the center of the cup. One ml of supernatant was placed on the left side of the Conway

barrier and 1 ml of saturated Na_2CO_3 solution was placed on the right partition. The cup was tightly closed and the vial cap was covered with vaseline before the cup was shaken for a few minutes so the supernatant mixes with NaOH. Then left for 24 hours at room temperature. Ammonia coming out of the supernatant due to react with Na_2CO_3 was immediately bound to boric acid. Ammonia bound to boric acid was titrated with H_2SO_4 0.005 N until a blue discoloration becomes reddish. The N-NH3 content was calculated by the following formula: NH3 = (ml Titration × N H_2SO_4 × 1000) mM.

The content of the dry matter and organic matter was analyzed. As blanks were used rumen fluid without treatment. The dry matter and organic matter digestibility coefficients are calculated by the equation:

$$\text{IVDMD (\%)} = \frac{\text{DM initial - (DM residue - DM blanko)}}{\text{DM initial}} \times 100$$

$$\text{IVOMD (\%)} = \frac{\text{OM initial - (OM residue - OM blanko)}}{\text{OM initial}} \times 100$$

Statistical Analysis

Data of the in-vitro rumen fermentation and digestibility parameters were analyzed by One Way - ANOVA using SPSS ver. 21 and Duncan Multiple Range Test (DMRT) was used to compared the treatments means [10].

RESULTS AND DISCUSSION

The effects of *E. hirta* supplementation in feed on rumen fermentation can be seen in Table-2.

Table-2: The effects of *E. hirta* supplemented in feed on the *in-vitro* rumen fermentation and digestibility.

Parameters	Treatments					
	P_0	**P_1**	**P_2**	**P_3**	**P_4**	**P_5**
Rumen pH	6.82±0.07	6.86±0.05	6.86±0.05	6.95±0.24	6.87±0.04	6.81±0.07
Total VFA (mM)	$71.28±0.37^a$	$82.91±0.94^b$	$88.53±0.48^c$	$97.50±0.72^d$	$72.05±0.02^a$	$71.32±0.30^a$
N-NH_3 (mM)	$9.28±0.13^b$	$10.64±0.46^c$	$11.70±0.33^d$	$13.86±0.19^e$	$9.04±0.36^b$	$4.81±0.60^a$
IVDMD (%)	$53.82±0.26^a$	$58.66±0.25^b$	$62.32±0.99^c$	$64.32±1.06^d$	$54.84±0.36^a$	$54.71±0.86^a$
IVOMD (%)	$58.37±0.35^a$	$61.07±0.32^b$	$63.01±0.80^c$	$64.38±0.77^d$	$59.19±0.87^a$	$58.96±0.79^a$

Different superscripts on the same line shows significant difference (P<0.05)

The effect of herbal supplementation in feed on rumen pH

High and low pH of the rumen fluid is a determining factor for whether or not the conditions for the process of rumen fermentation. Statistically, the supplementation of *E. hirta* in feed had no significant effect (P>0.05) on rumen fluid pH. The highest rumen fluid pH value was in P_3 treatment (6.95±0.24) and the lowest one was in P_5 (6.81±0.07) (Table-2). It indicated that there was no side effects of herbal addition into the rumen microorganisms and the in vitro rumen fermentation system. This pH value is ideal to support a good rumen environment for growing rumen microbe and digesting feed such as fiber and proteins.

The pH value of rumen fluid of goats fed with the supplementation of *E. hirta* in feed was still within the normal range of between 6.5-7.0, [11]. The activity of cellulotic bacteria was inhibited when the rumen fluid pH was below 6.2 and the microbial activity is optimal at pH 6.7±0.5 [12].

The results of this experiment related with the study done by [13] who found that betel leaf herb (*Piper betle* L.) added to feed did not affect the rumen pH. This might be caused by betel leaf containing bioactive compounds which are also owned by *E. hirta*, therefore it might provide the same effect on the rumen pH. The bioactive compounds contained in betel leaves were steroids, diterpenes, tannins, flavonoids, saponins, coumarin and alkaloids [14].

The effect of herbal supplementation in feed on total VFA rumen concentrations

The result showed that the supplementation of *E. hirta* in feed was highly significant effect (P<0.01) on total VFA concentration. The Duncan test showed that the highest concentration of total VFA was in P_3 (P<0.01), 97.50±0.72 mM, followed by treatments of P_2, P_1, P_4, P_5 and the lowest was on P_0 treatment, 71.28±0.37 mM (Table-2). The total VFA concentration among treatments of P_0, P_4 and P_5 showed not significant difference (P>0.05).

The high concentrations of VFA in the rumen of treatments of P_3, P_2 and P_1 showed that content of bioactive compounds in *E. hirta* given up to 15% (P_3). These did not cause fermentation by rumen microbes in degrading crude fiber (starch, cellulose, hemicellulose and pectin) until the formation of VFA so that the

concentration of VFA produced is still high. The high concentrations of VFA in the rumen also increased availability of energy for the growth of rumen microbes where VFA in the rumen is the main source of energy and the source of carbon skeletons for the formation of microbial proteins in rumen [15].

However, at high concentration of *E. hirta*, 20% (P_4) and 25% (P_5), the VFA concentration in rumen fluid decreased to 72.05±0.02 mM (P_4) and 71.32±0.30 mM (P_5). It looks the two treatments were not significantly different in comparison with control (P_0) with the concentration of VFA of 71.28±0.37 mM. The decrease of VFA concentrations in the rumen might be due to the high concentration of *E. hirta* in the treatment of P_4 and P_5 leading to higher tannin intake, which may affect to the decrease of rumen microbial activity. This might be due to excessive tannin properties to inhibit some rumen microbial ativity, particularly fibrolytic bacteria. According to [16, 17], many plant extracts and plant secondary metabolites at high doses might possibly reduce the total of VFA concentrations as well as partial VFA concentrations as a result of their antimicrobial effects.

Bioactive compounds within *E. hirta* such as tannins, saponins, flavonoids and alkaloids have antibacterial properties. Secondary metabolites such as tannins, saponins, flavonoids and alkaloids have antimicrobial effects that could increase the permeability of microbial cells membrane without destroying them, inhibiting microbial enzymes and inhibiting multiplication and microbial growth [18]. With the disruption of rumen microbial activity, the process of fermentation of crude fiber in the rumen by microbes converting into volatile fatty acid (VFA) was also disrupted, it therefore the concentration of VFA produced also decreased up to the amount which was not different from the control (P_0).

The VFA concentration of rumen fluid resulted in this study was still within the normal range as recommended by [19], it was about 70-150 mM. Total VFA production as a response of *E. hirta* supplemented in feed varied according to level of supplementation in feed. The high level of herbal supplementation in feed might reduce the population of rumen bacteria. On the other hand, the low level of herbal supplementation in feeds could modulate the bacterial growth leading to produce high VFA concentration. The bioactive compounds at low doses, they have the potential to improve rumen fermentation, but at high doses, they have an adverse effects on rumen fermentation [20].

The effect of herbal supplementation in feed on N-NH_3 rumen concentrations

The result showed that supplementation of *E. hirta* in feed was highly significant effect ($P < 0.01$) on N-NH_3 rumen concentration. The Duncan test showed that the highest concentration of N-NH_3 was found on treatment of P_3 ($P<0.01$), 13.86±0.19 mM, followed by the treatments of P_2, P_1, P_0, P_4 and the lowest was on P_5 treatment, 4.81±0.60 mM.

The higher concentration of N-NH_3 in treatment of P_3, P_2 and P_1 than those in control (P_0) showed that the content of bioactive compounds contained in *E. hirta* such as saponins, flavonoids, alkaloids and tannins given up to 15% level did not disturb the rumen microbial activity in changing protein to ammonia, so that N-NH_3 concentration resulted was still high. It also increased the availability of microbial protein-forming components in which ruminal N-NH_3 is the principal microbial N source to synthesize amino acids for their growth [21, 22].

However, on the higher content of *E. hirta* (20%) in treatment of P_4, N-NH_3 concentration decreased to 9.04±0.36 mM. Given 25% (P_5) *E. hirta*, N-NH_3 concentration decreased more to reach the lowest concentration, 4.81±0.60 mM. The decrease of N-NH_3 concentration by increasing *E. hirta* (25% in P_5), causes the intake of bioactive compounds, especially the tannins are higher in the rumen causing rumen microbial activity to be disturbed due to excessive tannins properties which could also poison some rumen microbes. As suggested by [23], tannins contain hydroxyl and carboxyl groups that can form complexes with bacterial cells membrane that may interfere bacterial activity. With the disruption of rumen microbial activity there is a decrease in feed protein degradation in the rumen to be converted into amino acids and ultimately into N-NH_3 ammonia, resulting in decreased N-NH_3 concentration.

The results of this research are related with the results found by [24] with the addition of sambiloto (*Andrographis paniculata*); [25] with the addition of lemongrass (*Cymbopogon citratus* Stapf.), and [26] with the addition of lemongrass or galangal in the diet, where herbal supplementation significantly ($P<0.05$) decreased the N-NH_3 concentration in rumen fluid. This is because the herbs contain bioactive compounds that are also owned by *E. hirta*, when given in high concentrations they can disrupt the activity of rumen microbes. The result also reduced the rate of proteolysis, peptidolysis and protein deamination by microbes so as to affect the decrease in N-NH_3 concentration produced.

The N-NH_3 concentration resulted in this research is still within the normal range, 4.81-13.86 mM. In accordance with the opinion of [27], the concentration of N-NH_3 which supports the growth of microbes in the rumen is about 4-14 mM. The ammonia concentration in rumen fluid varies depending on the amount of feed protein, protein degradation rate and time after feeding [28]. N-NH_3 concentrations less than the minimum limit of the normal range may interfere with the fermentation process. The decrease in ruminal

$N\text{-}NH_3$ concentrations is associated with a decrease in total VFA concentrations, which leads to a decrease in overall fermentation of feed [29], since VFA is a major source of energy for ruminants, a decrease in ruminal VFA production may have adverse nutritional consequences if this effect expressed through in-vivo.

The effect of herbal supplementation in feed on dry matter *in vitro* digestibility

Statistically, supplementation of *E. hirta* in feed showed highly significant effect ($P<0.01$) on the dry matter *in vitro* digestibility. The Duncan test showed that the highest dry matter digestibility was found in treatment of P_3 ($P<0.01$), 64.33±1.06%, followed by treatments of P_2, P_1, P_4, P_5 and the lowest was in treatment of P_0, 53.82±0.26% (Table-2). There was no significant effect among treatments of P_0, P_4 and P_5 ($P>0.05$).

The high dry matter *in vitro* digestibility in the treatment of P_1, P_2 and P_3 showed that bioactive compounds from *E. hirta* supplemented up to 15% in feed did not have a negative effect on rumen *in vitro* digestibility. The nutrient content of feeds such as crude protein and energy at the addition of 15% of *E. hirta* is sufficient enough for surviving of the microbes viability in the rumen and could increase the activity of rumen bacteria in the formation of cellulotic bacteria to degrade carbohydrates. The results of this study were consistent with the findings of [26] suggesting that the addition of some herbs to the diet does not negatively affect the growth and activity of most species of cellulotic bacteria.

However, giving high concentration of *E. hirta* in feed, the treatment of P_4 and P_5, decreased the dry matter *in vitro* digestibility which was no different from control (P_0). The decrease of dry matter *in vitro* digestibility in the rumen was due to the excess *E. hirta* in P_4 and P_5 treatments then leading to high intake of bioactive compounds present in the rumen, which could lead to decrease the number of rumen bacteria due to the nature of the bioactive compounds such as tannins that could bind bacterial cells membrane [23]. As a result, rumen microbes that degrade carbohydrates fermentatively also decreased and then followed by the decrease of the dry matter *in vitro* digestibility in the rumen.

Dry matter *in vitro* digestibility is one of indicators to determine the quality of feed. The higher the dry matter *in vitro* digestibility, the higher the opportunities of nutrition to be used by cattle for growth and production [30]. In-vitro digestibility of dry matter shows the proportion of dry matter feeds that could be digested by rumen microbes. According to [19] feed digestibility is influenced by chemical composition of feed and fibrous feed fraction.

The results of this research indicated that the average dry matter in vitro digestibility (57.72%) of *E. hirta* supplemented in feeds was higher than those results obtained by [24] using feed added with sambiloto (*Andrographis paniculata)* with the dry matter digestibility value of 45.33-55.00%, and by [13] using feed added with betel leaves (*Piper betle*) with the dry matter digestibility of 48.65-53.21%. This might be possibly because of betel and bitter leaf containing high bioactive compounds of antibacterial, thus decreasing the activity of rumen microbes in degrading dry matter feeds. As a result dry matter digestibility of feed supplemented by *E. hirta* were higher than those given sambiloto (*Andrographis paniculata*) and betel leaf (*Piper betle*).

The effect of herbal supplementation in feed on the organic matter *in vitro* digestibility

Statistically, supplementation of *E. hirta* in feed showed highly significant effect ($P<0.01$) on the *in vitro* digestibility of organic matter. The Duncan test showed that the *in vitro* digestibility of organic matter on treatment of P_3 was very significantly ($P<0.01$) highest, 64.38±0.77%, followed by treatment of P_2, P_1, P_4, P_5, and the lowest was at P_0 treatment, 58.37±0.35% (Table-2). There was no difference among P_0, P_4 and P_5 treatments.

Differences in the *in-vitro* digestibility of organic matter among treatments were caused by differences in dry matter *in-vitro* digestibility at each stage of supplementation. The increase of the *in-vitro* digestibility of organic matter on the supplementation of *E. hirta* in feed up to 15% level was caused by the increase of the dry matter *in vitro* digestibility, since the dry matter *in-vitro* digestibility is proportionally related to the *in vitro* digestibility of organic matter. The increase of the *in-vitro* digestibility of organic matter and the *in vitro* digestibility of dry matter might be due to the increase of microbial population and then the activity of microbes in the rumen as a result of the availability of adequate and balanced nutrition. It migh be also due to feeds having the high crude protein content that causes increased rumen microbial activity, organic matter digestibility and protein synthesis in the rumen and bioactive compounds in *E. hirta* have not disturbed the digestive process in the rumen in this case the digestibility of organic matter. In addition, organic matters (starch, cellulose and hemicellulose) would be fermented by microbes into the organic fermentation products such as acetic acid (C_2), propionic acid (C_3), butyric acid (C_4) and gas (CH_4 and CO_2) [31], thereby increasing the rumen VFA concentration and consequently increasing the digestibility of organic matter. The increased concentration of rumen VFA was closely related to the digestibility of organic matter [32].

The decrease of *in vitro* digestibility of organic matter in treatment of P_4 and P_5 might be

caused by the average of dry matter *in vitro* digestibility value in the treatment was also low as a result of too high concentration of *E. hirta* which were 20% and 25%. The decrease of the *in vitro* digestibility of organic matter might posibbly be suspected because the ability of microbes in receiving nutrients (bioactive compounds) exceeds the maximum limit, causing the decrease of microbial activity in rumen. The increase of the content of bioactive compounds such as tannins, which could bind rumen bacterial cells membrane thereby decreasing the activity of rumen microbes in degrading nutrients in feed. As a result, the dry matter *in vitro* digestibility of feed in the rumen decreased and also followed by the decrease of the *in vitro* digestibility of organic matter in feeds. Apart from tannin, this reduction might be also suspected because of the presence of saponins that could lysis on protozoan cell walls, and also negatively affecting the activity of rumen bacteria. One factor that affects the effectiveness of saponins against protozoa was the dose of saponins [33]. The decrease in the *in-vitro* digestibility of organic matter might also be caused by the alkalois compuond within *E. hirta* that could also interfere the bacterial activity. The tannins, saponins, flavonoids and alkaloids have antimicrobial effects that could increase the permeability of microbial cells membrane, inhibit microbial enzymes and inhibit microbial multiplication and growth [18].

The digestibility of organic matter is higher than dry matter digestibility value, this might be due to the dry matter still contained ash, whereas in organic matter does not contain ash, so that the material without the ash content is relatively easy to be digested. According to [15], the rate of degradation of organic matter was closely related to the rate of degradation of dry matter since most of the dry matter consists of organic materials, which distinguishes it only on ash content in feed materials.

CONCLUSION

Supplementation of *E. hirta* up to 15% based on dry matter in feed could affect the rumen environment such as the total VFA concentration and $N\text{-}NH_3$ concentration and the *in vitro* digestibility of dry matter and organic matter.

SUGGESTION

Further study is needed to explore the effect of supplementation of *E. hirta* in feed on the rumen microbial population, and in-vivo experiments to determine the effect of *E. hirta* in feed on the hematology and blood biochemistry aspect, and its effects on increasing production and milk quality.

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