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Chemical Quality of Rumen Fermentation And In Vitro Digestability Of Complete Feed Based on Sorgum-*Clitoria Ternatea* Silage with Additional Concentrate Contains Znso4 And Zn-Cu Isoleucinate

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ABSTRACT
This study was done to examine the chemical quality of rumen fermentation and *in vitro* digestibility of complete feed based on silage sorghum-*Clitoria ternatea* with the level of addition of concentrates. The Complete Randomized Disgn was used in this study with four treatments and four replications. The treatments were T_1: Sorghum silage - *Clitoria ternatea* without concentrate; T_2: T_1 + 10% concentrate; T_3: T_1 + 20% concentrate and T_4: T_1 + 30% concentrate. The concentrate contains 150 mg $ZnSO_4$/kg DM and 2% Zn-Cu isoleucine/kg DM ration. The parameters studied were 1). chemical quality; 2) production NH_3 and VFA *in vitro*; 3) digestibility of DM and OM (DDM and DOM) *in vitro*. The results showed that the treatment could be increased the crude protein content and the increase occurred linearly, but decrease the crude fiber content such as NDF, ADF, cellulose, and lignin, while the content of hamicellulose had no efeect. The treatments also had no effect on the pH value, decreased VFA production, and increased NH_3 production *in vitro*, but the highest contribution of protein and crude fiber to NH_3 and VFA production was at the level addition of T_1 treatment. Treatments had an effect on increasing DDM and DOM *in vitro* and the highest was at the level of addition of 10% concentrate (T_1). It could be concluded that the protein and crude fiber content gave a highest contribution to the production of NH_3, VFA, DDM and DOM *in vitro* at 10% concentrate level.

Keywords: Sorghum, *Clitoria ternatea*, ZnSO4, Zn-Cu isoleucine, NH3, VFA

INTRODUCTION
Forage feed is naturally needed for ruminants as a source of fiber and a source of energy for the synthesis and growth of rumen microorganisms. Therefore, if the nutrient needs for livestock are not met, it will have an impact on weight loss, even causing death (Suryana, 2008). To increase livestock productivity (production and population) quality, quantity and continuity of forage are required, especially during the dry season (Rubianti, *et al.* 2010). There are some superior plants that can be used as forage for livestock and not affected by the season like sorghum and telang flower (*Clitoria ternatea*) (Nullik, 2009).

Sorghum (Sorghum bicolor L) is a cereal crop that has great potential to be developed in Indonesia because it is tolerant of drought, waterlogging, can produce on marginal land and is more resistant to pests or diseases (Sirappa, 2003). In developed countries, sorghum is grown as animal feed because it contains a fairly high source of nutrients, i.e. 11.00% crude protein, 3.32 kcal energy, 73% carbohydrates, 3.30% fat, 2.30% fiber and Ca, P and Fe were 2.8, 2.87 and 4.40 mg respectively in 100g of material (Sirappa, 2003). The next researcher reported the nutritional content of sorghum in the vegetative phase containing crude protein 13.76%-15.66% with crude fiber 26.06%-31.81% (Purnomohadi, 2006). To increase the protein content of forage in its cultivation, it needs to be combined with legumes because it can increase the availability of soil nitrogen (Willey, 1990), so that it can overcome the shortage of fertilizer for farmers to increase forage production, one of which is flower telang (*Clitoria ternatea*) which can well adapt to the local environment in semi-arid areas and is quite successful when planted in an intercropping system with grasses such as elephant grass, corn and sorghum and so on with a fairly high production (Nullik, 2009; Jelantik, et al. 2015). Therefore this forage is the most prospective plant to be used as material basic animal feed due to high production and quality compared to other legumes (Jelantik et al., 2015). In terms of plant quality, *Clitoria ternatea* contains 16-18% protein, 18.6 MJ/kg crude energy, 69.7% organic matter digestibility, 66.6% energy digestibility and 12.4 MJ/kg metabolized energy in ruminants (Sutedi , 2013).

However, its use as feed is very limited caused by high tannin content, reaching 0.40-3.60% (Rooney and Sullines, 1977 in Sirappa, 2003). In an effort to increasethe benefits of sorghum, a breakthrough is needed to reduce tannin levels contained in it and one of the technological innovations that can be used is silage technology. To improve the quality of sorghum and overcome the scarcity of fertilizers, mixed planting is carried out with a sorghum intercropping system using *Clitoria ternatea* plants aimed at increasing soil productivity, due to the increasing amount of solar radiation energy emitted by the plant. Capable of being captured by the plant shoots (Sumarsono, 2008). The cropping pattern of mixed cropping or intercropping with legumes must be planted with the appropriate spacing (Sumarsono, 2009).

In the intercropping pattern with wide spacing and different root systems and with adequate fertilization, there will be no competition for both sunlight and nutrients (Efendi, et al. 2010). The results of the study Hartati *et al*. (2019[a]) showed that intercropping sorghum with *Clitoria ternatea* at a spacing of 40x40 cm was the ideal spacing in influencing the production and quality of forage produced. It was further reported that the silage of mixed sorghum-*Clitoria ternatea* at a spacing of 40x40 cm was the best silage indicated by *in vitro* DDM and DOM parameters and the highest total VFA concentration. Sorghum-*Clitoria ternatea* silage supplemented with a concentrate containing 150 mg ZnSO4/kg DM and 2% Zn-Cu isoleucine/kg DM ration (best results Hartati et al. 2009[a] study) was given to goats and produced the highest fermented products, DDM, DOM, weight gain at the level of 30% (Hartati et al, 2019[b]). Increased body weight is due to increased alkaline phosphatase activity which plays a role in energy metabolism. In addition, it is possible that the activity of the carboxypeptidase enzyme that plays a role in rumen microbial protein synthesis increases. Therefore, this study was designed to investigate the chemical quality of rumen fermentation and *in vitro* digestibility of complete feed based on sorghum-*Clitoria ternatea* silage with the addition of concentrate levels containing 150 mg ZnSO4 /kg DM and 2% Zn-Cu isoleucine/kg DM rations.

MATERIALS AND METHODS

At the preparatory stage, starting from land preparation, initial fertilization with bokasi fertilizer, then planting *Clitoria ternatea* seeds at a distance of 20x20 cm in April 2021 and after the seeds grow, they are immediately watered. Two weeks after the seeds began to germinate, then the sorghum seeds were planted at a distance of 40x40 cm. Urea fertilizer as a source of N was prepared in one kg/100 liters of water and then given in the morning after the sorghum plants were two weeks old using a 200-220 ml kocor system around the plant stems and then watered every two days.

Before the plants were cut, Zn-Cu isoleucine was prepared using the Hartati method (Hartati et al. (2009b) and prepared a concentrate with a formula based on local ingredients (corn flour, rice bran, coconut meal, fish meal, coconut oil, salt and premix), with the addition of concentrate 150 mg ZnSO4/kg DM and 2% Zn-Cu isoleucine/kg DM ration (Hartati et al., 2009b).

Furthermore, the mixture of sorghum-*Clitoria ternatea* was cut before the flowering phase at the age of 70 days after planting, then withered for 4-6 hours, 2-4 cm long was chopped. The forage that had been chopped was mixed homogeneously with a concentrate containing 150 mg ZnSO4/kg DM and 2% Zn-Cu isoleucine/kg DM ration. The concentrate was added based on the dry matter (DM) of the forage.

In the implementation, the research used an experimental method with a completely randomized design (CRD) 4 treatments were repeated four times.The four treatments investigated were T_1: Sorghum Silage-*Clitoria ternatea* without concentrate; T_2: T_1+ 10% concentrate; T_3: T_1 + 20% concentrate and T_4: T_1 + 30% concentrate. After that, each treatment was weighed one kg in size and put into a plastic silo, repeated four times for each treatment and the ensilation process during for 21 days.

After 21 days, the fermentation results were in the form of jam, followed by sampling for analysis in the Feed Chemistry laboratory, Faculty of Animal Science, Undana. Determination of dry matter (DM) was carried out by drying in an oven at 105°C until it reached a constant weight. The chemical composition of silage was determined using proximate analysis using the AOAC method (2005).

Crude protein content (CP) after acid-base extraction and fiber fractions (ADF, NDF, cellulose, hemicellulose and lignin) were determined using the AOAC method (2005). In determining the concentration of NH_3 *in vitro*, it was analyzed using the micro-diffusion technique (Conway, 1957), the total concentration of VFA *in vitro* was determined by the steam distillation technique (General Laboratory Procedure, 1996) and DDM and DOM *in vitro* were determined by the method of Tilley and Terry (1963). A total of 50g of silage samples were taken from each silo to determine the chemical composition of rumen fermentation to determine the concentration of NH_3, the concentration of VFA. Fresh rumen fluid was taken from the abattoir in the morning as inoculum. Put 0.5g of each sample into 100ml fermenter tube, add 40ml McDougal solution and 10ml rumen fluid. To create anaerobic conditions, CO_2 was added to each tube for 30 seconds. Then the tube was put into a shakerbath and fermented for 24 hours and added 2 drops of saturated $HgCl_2$ to kill the microbes so that the fermentation stopped. Then it was centrifuged at 5000 rpm for 30 minutes and the supernatant was taken for analysis of the concentration of NH3 fan VFA.

In determining DDM and DOM *in vitro*, two stages were carried out, i.e the first stage, fresh rumen fluid was taken from the abattoir in the morning as inoculum. Put 0.5 g of each sample into a 100 ml fermenter tube, add 40 ml of McDougal's solution and 10 ml of rumen fluid. To create anaerobic conditions, CO2 was added to each tube for 30 seconds. After being incubated for 48 hours, add 2-3 drops of pepsin HCl 0.2% to kill microbes. Then centrifuged at 5000 rpm for 15 minutes, the supernatant was discarded. The second stage after the supernatant was removed, the precipitate was added to 50 ml of 0.2% pepsin HCl solution and then incubated again for 48 hours under aerobic conditions. The remains of digestion were filtered through Whatman filter paper no. 41 then put in an oven at 105 oC and after 24 hours removed, weighed to determine the levels of BK. Furthermore, the material is put into a 600oC kiln and after 6 hours is weighed to determine the level of OM. For the blank, untreated rumen fluid was used. All data were analyzed by statistical analysis using Proc. GLM (SPSS version 21) and to determine the difference between treatments, Duncan's test was applied.

RESULTS AND DISCUSSION

Chemical Composition of Complete Feed

The average chemical composition of complete feed based on sorghum silage (PKSS-Ct) without concentrate (T1) and with the addition of concentrate contains 150 mg ZnSO4/kg BK concentrate and 2% Zn-Cu isoleucine/kg DM ration at the level of 10% 20% and 30% (T2, T3and T4) are presented in Table 1.

TABLE 1: Chemical Composition of Research Ration

Chemical Composition	Treatments				P-value
	T_1	T_2	T_3	T_4	
Dry matter (%)	25,82±3,37[a]	33,89±2,49[b]	40,81±2,66[c]	41,01±1,35[c]	<0,000
Organic matter (%)	78,97±5,58[a]	79,12±1,59[a]	84,92±2,17[b]	84,96±1,49[b]	0,016
Crude protein (%)	15,28±0,35[a]	17,84±1,03[b]	19,27±1,04[c]	21,13±0,51[d]	<0,000
Crude fiber (%)	24,38±0,26[a]	18,97±1,91[b]	13,23±0,67[c]	11,86±1,00[c]	<0,000
Crude fat (%)	7,45±1,10a	13,62±1,10[b]	13,95±0,61b	14,60±0,25[b]	<0,000
CHO (%)	56,24±4,65[a]	47,66±1,92[b]	51,70±1,12[c]	49,22±2,01b[c]	0,004
NFEM (%)	31,87±4,77[a]	28,69±2,55[a]	38,47±1,57[b]	37,36±1,58[b]	0,002
GE (kcal/kg)	3803,66±271,04[a]	4099,21±54,79[b]	4374,93±117,55[c]	4433,27±53,05[c]	<0,000
EM (kcal/kg)	2602,82±285,33[a]	3094,99±94,37[b]	3586,05±122,23[c]	3666,40±43,77[c]	<0,000
Fiber fractions					
NDF (%)	54,32±1,34[a]	50,46±0,72[b]	47,72±0,90[c]	45,75±0,90[d]	<0,000
ADF (%)	24,49±2,09[a]	18,69±0,98[b]	15,81±0,92[c]	13,89±0,85[d]	<0,000
Cellulose (%)	16,76±0,83[a]	14,77±0,81[b]	10,98±0,78[c]	9,32±0,66[d]	<0,000
Lignin (%)	7,72±1,46[a]	5,23±0,84[b]	3,22±0,57[c]	2,41±0,38[c]	<0,000
Hemicellulose (%)	29,83±1,56[a]	31,77±0,34[b]	31,91±1,16[b]	31,85±1,21[b]	0,060

Note: **The results of the analysis of the feed chemistry laboratory, Faculty of Animal Husbandry, Undana
Different superscripts in the same row showed significant differences (P<0.05)

Table 1 showed that there has been a linear increase in protein content from treatment T_1 to T_4. The increase in protein content can be understood because the protein concentrate content is quite high, so the higher the concentration level added, the higher the protein content. The results of statistical analysis showed that the treatment had a very significant effect ($P<0.01$) on increasing the protein content of the complete feed. This was due to differences in the level of concentrates containing 150 mg ZnSO4/kg BK concentrate and 2% Zn-Cu isoleucine/kg BK rations in the manufacture of complete feeds. These results showed that the crude protein content in the T_2 treatment gave the highest contribution to the increase in crude protein. This is because in addition to the crude protein content of *Clitoria ternatea* plant, it is higher than forage sorghum by 14-20%, while fresh *Clitoria ternatea* leaves contain 21.4% crude protein and 29% crude fiber (Kalamani and Gomez, 2001). If *Clitoria ternatea* is in the form of hay cut at the age of 42 days, the crude protein content is 34.84% and crude fiber is 28.94% (Barro and Ribeiro, 1983 in Setiadi, 2013). Research results Hartati et al. (2019b) the crude protein content of sorghum-*Clitoria ternatea* silage with a spacing of 40 x 40 cm was 19.22%.

Table 1 also shows that the crude fiber content from treatment T_1 to T_4 decreased linearly and was lowest in treatment T_4. This means that the T_4 treatment gives the highest contribution to the decrease in crude fiber content and will have an impact on increasing digestibility. However , the results of the study showed a decrease in DDM and DOM *in vitro* in the T_3 and T_4 treatments and this was related to the NH3 production which also decreased in the T_3 and T_4 treatments . The results of statistical analysis showed that the treatment had a high significant effect ($P<0.01$) on the decrease in crude fiber content and the lowest decrease was in the T_4 treatment. Furthermore, the decrease in DDM and DOM was followed in treatment T_3 and T_2 compared to T_1 respectively. This is because the crude fiber content in the concentrate is low in addition to forage sorghum-*Clitoria ternatea* which is cut before flowering results in low crude fiber and lignin content.

It is well known that crude fiber is the part that is difficult to digest which consists of cellulose, hemicellulose and lignin which is a limiting factor for the growth and activity of microorganisms in the rumen. Cellulose is a fundamental structure in plant cell walls (McDonald et al. 2002), while hemicellulose is a cell wall fraction that is soluble in alkali as polysaccharides. Meanwhile, lignin is a non-carbohydrate fiber fraction and is very resistant to chemical degradation. In this study, after the T_1-T_4 treatment feed underwent a fermentation process in the manufacture of silage, there was a linear decrease in lignin levels and the lowest was in the T_4 treatment (Table 1). The results of statistical analysis showed that the treatment had a very significant effect ($P<0.01$) on the decrease in lignin content and the lowest lignin content in treatment T_4 and the decrease was not significantly different ($P>0.05$) with treatment T_3 ($P>0.05$), however, the two treatments were significantly different ($P<0.05$) from the T_2 treatment. This is because the more concentrate added to the complete feed, the lower the crude fiber content followed by the lower lignin content.

In ruminants, crude fiber is used as an energy source and will affect livestock growth. The fiber fraction consists of NDF which is easily soluble in neutral detergents and is the largest part of plant cell walls and this material consists of cellulose, hemicellulose, lignin, silica and fibrous protein (Van Soest, 1982). NDF degradation is higher than ADF because it contains a soluble fraction, namely hemicellulose and an increase in NDF content can reduce dry matter digestibility. While ADF is a material that is insoluble in acidic detergents consisting of cellulose, lignin and silica and the part that is easily digested is cellulose (Church and Pond, 1988). The lignin content of plants does not include carbohydrates and is a part that is difficult to digest and if the lignin content in the feed is high, the digestibility coefficient of the feed is low (Sutardi, et al. 1980).

In this study, the content of NDF, ADF, lignin and cellulose decreased linearly to T_4. The results of statistical analysis showed that the complete feed treatment based on sorghum-*Clitoria ternatea* silage with the addition of concentrate had a very significant effect ($P<0.01$) on the decrease in the content of NDF, ADF, lignin and cellulose. The lowest decrease was in the T_4 treatment. This happened because the crude fiber content of forage sorghum harvested before flowering was still low, namely

24.38%, in addition to the low content of concentrated crude fiber. On the other hand, the hemicellulose content increased in the T_4 treatment.

The content of NDF and ADF can be utilized by ruminants due to the activity of microorganisms in the rumen. Microorganisms produce enzymes that can degrade crude fiber to produce VFA as an energy source and carbon skeleton (C) for microbial protein synthesis. The results of Hartati et al (2019b)'s research showed that mixed planting with a distance of 40 cm x 40 cm was able to reduce the crude fiber content of forage. This is due to the wide spacing and different root systems and adequate fertilization, and there will be no competition for sunlight or nutrients. Populations of adjacent plants do not cause competition if the available water, soil, nutrients and solar radiation are at sufficient levels for each plant. According to (Keraf et al., 2015) that increasing plant age causes plants to enter a renesance phase where plants are already in a period of aging, causing plant parts to contain high cellulose and lignin. In this study, sorghum and *Clitoria-ternatea* were cut before flowering, so that the crude fiber content of T_1 was still quite low at 24.38%.

The NFEM content increased in the T_3 and T_4 treatments. The results of statistical analysis showed that the treatment had a very significant effect (P<0.01) on the increase in NFEM content.

This is because the content of NFEM which consists of monosaccharides, disaccharides and trisaccharides is relatively the same. According to Tillman et al. (1991) BETN consists of monosaccharides, disaccharides, trisaccharides and polysaccharides, especially those that are easily soluble in acid and alkaline solutions in crude fiber analysis which have high digestibility. It is known that NFEM is a source of C framework along with nitrogen (N) derived from feed protein used for protein synthesis of rumen microorganisms. The results showed that the NFEM content in the 20% and 30% concentration addition treatment (T_3 and T_4) was higher and between T_3 and T_4 was relatively the same compared to the 10% (T_2) and no concentrate (T_1) levels. This condition will result in differences in VFA concentrations.

Effect of Treatment on Acidity Level (pH), Fermentation in Rumen and Digestibility of Dry Matter and Organic Matter *in vitro*

Fermentation products in the form of concentrations of NH3 and VFA in the rumen, respectively, are used as a source of N and C framework and energy for protein synthesis of rumen microorganisms, the production of which is strongly influenced by the level of acidity or pH of the rumen fluid. The average values of pH, fermentation products (NH3 and VFA concentrations), DDM and DOM *in vitro* in the complete feed treatment based on sorghum-*Clitoria-ternatea* silage with the addition of concentrate in all treatments are presented in Table 2.

TABLE 2: Average pH Value, Fermentation Products (NH3 and VFA) *in vitro* Complete Feed Silage Sorghum-*Clitoria ternatea*

Parameters	Treatments				P-value
	T_1	T_2	T_3	T_4	
VFA (mM)	$121{,}25 \pm 4{,}09^a$	$134{,}49 \pm 5{,}87^a$	$131{,}89 \pm 4{,}14^a$	$131{,}67 \pm 9{,}71^a$	0,091
NH3 (mM)	$11{,}30 \pm 1{,}12^a$	$13{,}93 \pm 2{,}84^a$	$12{,}35 \pm 1{,}74^a$	$11{,}86 \pm 2{,}49^a$	0,460
DDM (%)	$65{,}50 \pm 3{,}64^a$	$68{,}12 \pm 1{,}01^a$	$60{,}99 \pm 1{,}74^b$	$60{,}93 \pm 3{,}95^b$	0,002
DOM (%)	$60{,}09 \pm 3{,}05^a$	$64{,}43 \pm 1{,}70^b$	$58{,}40 \pm 2{,}69^a$	$57{,}42 \pm 3{,}81^a$	0,004
pH	$6{,}56 \pm 0{,}05^a$	$6{,}58 \pm 0{,}38^a$	$6{,}57 \pm 0{,}03^a$	$6{,}55 \pm 0{,}09^a$	0,881

Note: Different superscript in the same row indicate significant differences (P < 0.05)

The rumen pH value in this study was relatively the same, namely 6.56-6.58 in all treatments. According to Van Soest (1982) this range of pH values provides a good environment for optimizing the digestibility of crude fiber (DCF). The results of this study indicate that the pH values for all treatment levels are relatively the same, and the values obtained are still within the limits where the fermentation process runs optimally.

In Table 2 it can be seen that there has been an increase in the average concentration of NH_3 in the treatment with the addition of concentrate to the level of 30%. The results showed that the treatment had no significant effect (P>0.05) on the increase in NH_3 concentration. This means that the level of increase in the concentration of NH_3 is relatively the same in all treatments with an average value between 11.30-13.93 mM/l and the highest NH_3 concentration is at the level of 10%. Because the concentration of NH_3 is closely related to the protein content of the feed and in this study, the highest protein content was at the addition of concentrate level of 30% (T_4), while the highest concentration of NH_3 was at the level of 10% (T_2). Thus, the highest contribution of protein to the NH_3 concentration was in the 10% concentration addition treatment. This is because most of the protein content of feed in the fermentation process will undergo hydrolysis into peptides, amino acids, ammonia and amines, then ammonia is rapidly converted to NH_3 (Hartati et al. 2019a).

The ammonia together with carbohydrates as a source of carbon (C) skeleton and energy form rumen microorganism proteins. It is possible that at the level of adding 20% and 30% concentrates (T3 and T4) the conversion of ammonia to NH_3 is very high, so that ammonia poisoning has occurred because the fermenter tube is closed, so that the population of microorganisms decreases and subsequently affects the decrease in NH_3 concentrations.

The results of the study on the concentration of VFA were the same as the effect of treatment on the concentration of NH_3, there was an increase in the concentration of VFA at all levels of addition of concentrate (T_2-T_4 vs T_1) and the concentration of VFA ranged from 121.25-134.49 mM/l, but the increase was not significant. (P>0.05), meaning that the effect of treatment at all levels is relatively the same. In this study, both the NH3 concentration and the VFA concentration were still in the normal range to support the fermentation process in the rumen. Naswantara (2006) reported that goats that consumed rice straw supplemented with protein and energy sources showed VFA concentrations reaching 71.81 mM/l. While McDonald et al. (2010) reported the concentration of VFA in ruminants between 70-150 mM/l. It means that the level and rate of fermentation in the rumen in this study was quite high.

The fermentation process has an impact on the digestibility value of DDM and DOM and in this study it was seen that there was an increase in the value of DDM and DOM at the level of 10% concentrate addition, while for the addition of 20% and 30% concentrate levels there was a decrease. The results showed that the treatment had a very significant effect ($P<0.01$) on the increase in DDM and DOM values. The highest increase in the addition of concentrate at the level of 10% (T_2). This is because the protein, crude fiber and energy content at these levels are sufficient to produce concentrations of NH_3 and VFA which can further increase the synthesis of rumen microorganisms, so that the rate of fermentation in the rumen goes well. Furthermore, there was an increase in DDM and DOM and the highest was at the addition of 10% concentrate level.

CONCLUSION

It was concluded that there was an increase in protein content and a decrease in crude fiber content in complete feed based on sorghum-*Clitoria ternatea* silage with the addition of a concentrate containing 150 mg ZnSO4/kg DM ration and 2% Zn-Cu isoecinate/kg DM ration linearly. The rumen pH value was relatively the same in the four treatments, ranging from 6.56-6.58, but still within the limits of the optimal fermentation process. Protein content and crude fiber content gave the highest contribution to the NH_3 and VFA concentrations *in vitro*, respectively, with the addition of 10% concentrate. The highest concentrations of NH_3 and VFA *in vitro* resulted in the highest increase in DDM and DOM *in vitro* as well as the addition of 10% concentrate.

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