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***In vitro* study of *Nigella sativa* meal as protein source and its combination with the readily available carbohydrates for ruminant diet**

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Abstract. *Nigella sativa* meal (NSM) is a waste generated from the oil processing industry of black cumin or known as habbatussauda. NSM contains high crude protein that can be used by ruminant. The purpose of this study was to evaluate the quality of crude protein of NSM in rumen and its combination with the readily available carbohydrates (RAC). This study was began by observing ammonia (NH₃) concentration from NSM and volatile fatty acid (VFA) concentration from several types of RAC that were incubated in rumen fluid in vitro. Then followed by evaluation of rumen fermentability of diets containing NSM that was combined with different types of RAC. This study used a complete randomized design with 3 treatments and 5 replications. The treatments were differentiated by the type of RAC that was used in the diet, which consisted of pollard (P), rice bran (R), and corn (C). The level of NSM is equated in each treatment. Diet consisted of forage and concentrate with the ratio of 30:70. The results showed that NSM has high NH₃ concentration in the rumen, especially in the first 4 hours and after 6 hours of incubation time in rumen fluid in vitro. The combination of NSM in rations containing corn significantly suppressed NH₃ concentration ($P < 0.05$). In addition, NSM and its combination with corn in the diet produced microbial protein synthesis higher (9.66 mg 10ml⁻¹) compared with another treatments R (9.64) and P (7.85). The use of NSM as much as 20% in rations and its combination with the RAC derived from corn can optimize the use of NSM as a source of feed protein in ruminants by maximize the utilization of NH₃ and VFA to encourage the production of microbial protein synthesis that were important for ruminants.

1. Introduction

The exploration and diversification of feed ingredients becomes ones of essential topics for substituting the main feed commonly used in animal diets. This is one of the efforts that can be taken to eliminate dependence on a feed ingredient, especially feed protein sources with highly prized. Black seed (*Nigella sativa*) or known as habbatussauda or black cumin in the group of *Ranunculaceae* family is well known as herbal plants. *N. sativa* contain pharmacological properties which has been used as a medicine for various diseases. Pharmacological activity in *N. sativa* is related with the presence of thymoquinone compounds as the main compounds contained in oil which was found in *N. sativa* seeds [1]. Habbatussauda processing industry produces waste in the form of extraction residue of habbatussauda oil known as *Nigella sativa* meal (NSM). The use of habbatussauda in large and medium industries in Indonesia reached 144 817 kg year⁻¹ [2]. The potency of NSM produced is 101



372 kg year⁻¹ or as much as 70% of total raw materials [3]. NSM derived from habbatussauda oil processing residues taken using pressing method contain 8.1% water content, 23.3% crude protein, and 9.6% ash [4]. NSM also contains some minerals such as Mg, Fe, Cu, Ca, and K [5].

The use of NSM waste in Indonesia as feed ingredients is still small. Though NSM has the potential to be used as a source of feed protein because of the high crude protein content in it. In ruminant, when feeding the protein source into the rumen, the protein will be degraded into ammonia (NH₃), then the ammonia is brought into the bloodstream to the liver to be converted into urea. The lack of ammonia production in the rumen can suppress the flow of microbial protein synthesis from the rumen [6]. The excess ammonia production can increase blood ammonia levels, increase urine urea synthesis, and reduce the nitrogen retention of cattle [7]. In previous study reported that the use of NSM as much as 10 and 20% in the feed significantly improved the performance of lambs [8]. This indicates that NSM has good nutrient content, so it can improve the performance of lambs. However, the utilization of 10 and 20% NSM in feed significantly increases levels of blood urea nitrogen (BUN) [9]. The high levels of BUN indicate that NSM added in the diet causes an increase in protein degradation in the rumen. The high degradation of feed protein in the rumen may result in inefficiency because of the amount of protein degraded before it reaches the intestine to be utilized by the whole body [7].

Several ways are done to maintain and optimize the quality of feed, especially sources of feed protein for its usefulness in the feed material. One way that can be done is to combine source of feed protein with readily available carbohydrate (RAC). RAC will spur the growth of microbes in the rumen which also leads to high use of ammonia for microbial growth so that the potential ammonia that is converted into urea will be reduced [10]. Ideal conditions occur when the production of feed ingredients of fermented energy sources in the rumen is as fast as the production of NH₃, so that when NH₃ was formed there is volatile fatty acid (VFA) derived from carbohydrates to be used as the source and carbon chain of the amino acid microbial protein. The purpose of this study was to evaluate the quality of the crude protein of NSM in rumen in vitro and its combination with different types of readily available carbohydrates.

2. Material and Methods

The first stage of the research was analyze the rate of degradation of single feed. The rate of degradation observed as the concentration of NH₃ and VFA which described the rate of degradation of proteins and carbohydrates in the rumen. The next stage of the research was analyze degradation of feeds that have been formulated according to nutrient requirements of 5-month-old sheep in National Research Council [11]. Four experimental diets were prepared for this study. Diet contained 20% NSM (% DM) that was combined with pollard (P), rice bran (R), and corn (C), respectively. Forage and concentrate feeds were given at a ratio of 30:70. Preparation of treatment ration starting from the preparation of raw materials consisting of pollard, corn, onggok (tapioca by-products), rice bran, NSM, molasses, premix, and CaCO₃. Before all the ingredients were mixed, NSM was milled using a grinder with die diameter size of 6 mm. Then, the feed material was weighed according to the formulation in Table 1 and mixed manually. Mixing begins by including large quantities of ingredients. As for premix and CaCO₃ mixed first separately, then mixed with the carbohydrate source in the same volume with the mixture of minerals. This was done so that the mineral mixing is evenly distributed. The napier grass used in the ration mixture was dried for 2 days in oven 60 °C.

The feed is weighed into 0.5 gram fermentor tube (Pyrex glass tube with diameter 3 cm with height 20 cm) with ratio forage and concentrate 30:70. Then the feed was incubated in rumen fluid and McDougall solution [12]. The rumen fluid comes from a 1-year-old male sheep taken by a stomach tube. The fermentor tube is incubated in a waterbath shaker with a temperature of 39 °C. After 4 hours of incubation, the fermentor tube was removed from the shaker waterbath and was measured its pH. The liquid from each tube is stored in two different places, one for microbial protein synthesis (MPS) analysis and the remainder for analysis of NH₃ and VFA concentration. The samples for NH₃ and VFA concentration analysis were given two drops of HgCl₂ to kill microbial activity. Then the MPS

analysis is done according to [13] and the calculation of microbial N content using Lowry's method [14]. The production of NH_3 was analyzed by Conway method [15] and VFA with steam distillation method.

This experiment used a complete randomized design with 3 treatments and 5 replications. Data obtained from this study were statistically analysed by ANOVA (Analysis of Variance) by IBM SPSS Statistics. Duncan's multiple range tests was used to test the significance among means ($P < 0.05$) [16].

Table 1. The composition of the feed ingredients in each treatment.

Ingredients	P (%)^a	R (%)^b	C (%)^c
Napier grass	30.00	30.00	30.00
Pollard	19.60	0	0
Rice bran	0	31.50	0
Corn	0	0	24.50
Onggok (tapioca by-products)	19.95	8.05	15.05
NSM	19.60	19.60	19.60
Molasses	6.30	6.30	6.30
CaCO_3	0.70	0.70	0.70
Premix	0.35	0.35	0.35
CPO (crude palm oil)	3.50	3.50	3.50
Nutrient content (%DM)^d			
Dry matter	89.63	90.46	89.71
Ash	8.72	13.12	7.87
Crude protein	15.70	15.68	15.66
Crude fat	2.80	6.26	3.90
Crude fiber	16.46	17.70	13.41
Nitrogen free extract	56.32	47.24	59.16

^a P: NSM combined with RAC pollard

^b R: NSM combined with RAC rice bran

^c C: NSM combined with RAC corn

^d Estimated results of calculations based on the nutrient content of each ingredient

3. Results and Discussion

3.1. *In vitro* degradation rate of NSM

Degradation is a chemical change reaction or decomposition of compound or molecule into simpler form. Degradation rate of feed ingredients *in vitro* describes the time spent by the rumen microbes to decompose the nutrients present in a feed ingredient in an environment that has been designed similar with the digestive process that occurs in the ruminant digestive tract. The results showed that NSM has a fluctuating rate of ammonia degradation. At the fourth hour of incubation, ammonia concentration was higher than the previous hour, because the fourth hour of incubation is the optimal time for the rumen microbes to degrade the nutrients that enter into it. The maximum production of ammonia is achieved when 2-4 hours after feeding [17], so ammonia concentration in the rumen indicate how efficiently the protein digestion process is in the rumen [18]. The presence of protein and non-protein nitrogen (NPN) that enter to rumen causes the protein and NPN to be degraded by rumen microbes to NH_3 , peptides, and amino acids so that they will form microbial proteins. NH_3 is combined into amino

acids and finally formed into microbial proteins by rumen microbes [19]. Ammonia levels needed to support microbial growth are between 4-12 mM [17].

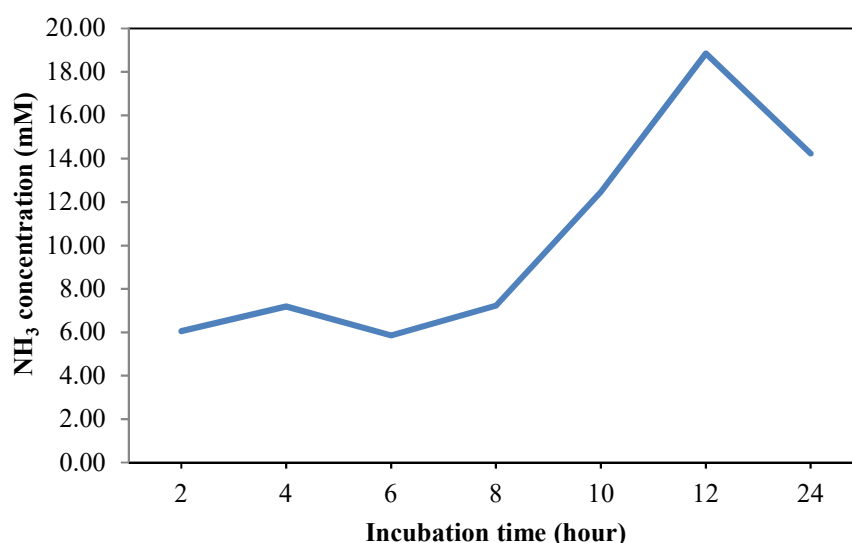


Figure 1. The ammonia concentration that measured over different incubation time of NSM.

At sixth hour incubation, NH₃ production decreased due to the use of NH₃ by rumen microbes for the purpose of microbial protein synthesis. Optimizing the growth of rumen microbes not only requires sufficient N availability, but other nutrients are also needed, such as energy, amino acids, minerals and vitamins. In the 8th to 12th hour, there is an increase in ammonia production. An increase in rumen microbial population driven by increased rumen microbial protein synthesis in the previous hours caused a comparable increase in protein degradation or NPN to ammonia. However, this ammonia cannot be used again to form microbial protein synthesis due to the lack of energy availability, so that ammonia accumulates in the Conway dish and its concentration will increase. The energy needed for microbial protein synthesis is energy in the form of ATP, while VFA which is beneficial for rumen microbes is only branching and is needed as a source of carbon framework. Branched chain amino acids strongly support microbial protein synthesis because they will undergo deamination and decarboxylation to produce branched chain fatty acids. At the 24th hour, the concentration of ammonia decreases may be caused by ammonia accumulation that has reached its maximum level, making it toxic to the rumen microbes which then suppresses the rumen microbial population, so that the process of protein or NPN degradation to ammonia also decreases. The optimal NH₃ concentration in the rumen is between 6-21 mM [20].

3.2. *In vitro* degradation rate of several types of RAC into VFA

Feed ingredients for carbohydrate sources in feed can increase microbial metabolic activity, both in degrading protein and carbohydrates. The end product of carbohydrate degradation will produce acidic compounds, such as acetate, propionate, and butyrate which will then reduce the rumen pH. The level of acidity that is too low will inhibit microbial growth, so that it will interfere with the food degradation process. Observations regarding the rate of carbohydrate degradation in Figure 2 show that each feed material has a rate of degradation to produce carbohydrates in different concentrations. In all types of RAC there was an increase in VFA concentration at the six hour incubation. Corn kernels consisted of starch (61 to 78%, dry matter (DM)), polysaccharides instead of starch (about 10%, DM), protein (6 to 12%, DM), and lipids (3 to 6%, DM) as a major component [21, 22]. Starch is the main component of the normal corn kernel, and is generally located in the endosperm (98% to 99% of total starch [21]).

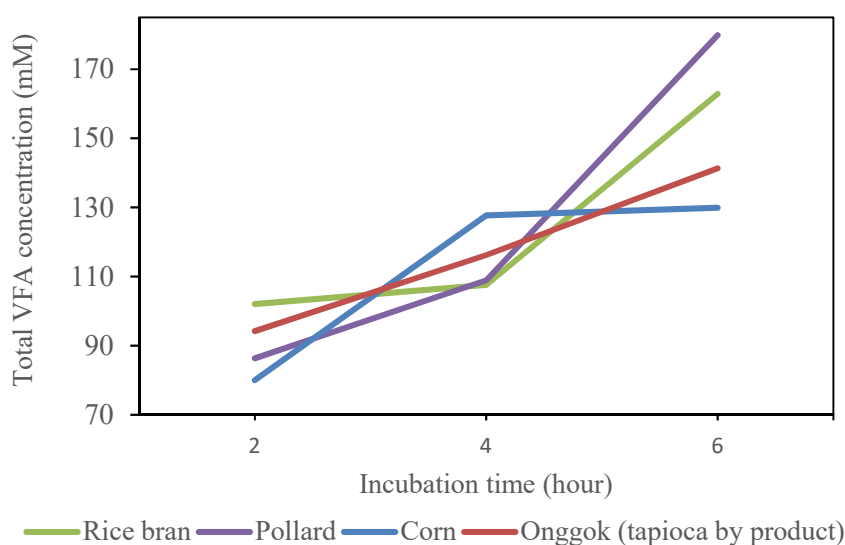


Figure 2. The VFA concentration that measured over different incubation time of several RAC.

Normal starch consists two types of α -D-glucopyranose polysaccharides: amylose and amylopectin. Amylose is basically a linear polymer that is connected by α (1 \rightarrow 4) glycosidic bonds with several α (1 \rightarrow 6) branch bonds, whereas amylopectin is a high branched polymer with about 5% α (1 \rightarrow 6) branch relationships [23]. Amylose content and branch chain structure of amylopectin from corn starch also affect its digestion rate. The digestive level of starch, in general, decreases with increasing amylose content and the length of the branch chain of amylopectin [24]. Corn contains 71-73% amylopectin and 22-27% amylose [25]. The cassava contains 83% amylopectin and 17% amylose, wheat contains 74% amylopectin and 26% amylose. Rice bran contains 5.66% amylose [26]. The source of rice bran used in this study may come from low-amylose rice varieties in Taiwan. Other factors, such as surrounding by cellulose and hemicellulose fibers and / or protein matrix and interact with lipids, can also reduce the susceptibility of starch to enzyme hydrolysis [27].

The difference in the content of amylose and amylopectin in the four types of materials used and the different content of cellulose and hemicellulose in them causes differences in the rate of starch degradation by rumen microbes. According to [10] easily fermented carbohydrates will stimulate microbial growth in the rumen which also causes high use of ammonia for microbial growth so that the potential for ammonia to be converted into urea will be reduced. The ideal condition occurs when the production of fermented energy source feed ingredients in the rumen is as fast as NH_3 concentration, so that when NH_3 is formed there is a VFA derived from carbohydrates that will be used as a source and carbon framework of microbial protein amino acids.

3.3. *In vitro* study of NSM and its combination with several types of RAC

NSM is an alternative source of protein. The results of previous study about degradation rate of NSM was indicated that NSM is rapidly degraded in the rumen, especially in the first 6 hours of fermentation in the rumen. The source of protein that enters the rumen will be degraded to ammonia and if this degradation process occurs quickly, it will cause inefficiency of protein use. Efforts that can be made to protect protein for ruminant animal feed are combine it with feed ingredients with a high content of RAC, because this RAC will be an energy source for rumen microbes which causes the use of ammonia in the rumen by microbes will increase, thereby reduce accumulation ammonia in the rumen. Ammonia will be used by rumen microbes to form microbial protein synthesis. Rumen microbes contribute considerable protein to the needs of ruminant animals [28]. This MPS is also an

important protein with high biological value in ruminants. The formation of the MPS also requires the supply of carbon branch chains derived from carbohydrates, and also requires vitamins and minerals.

Table 2. Production of NH₃, VFA, and microbial protein synthesis in each treatments.^a

Variables	P ^b	R ^c	C ^d
pH	6.84±0.09	6.96±0.09	6.94±0.11
NH ₃ (mM)	4.94±0.50 ^a	4.02±0.71 ^b	2.25±0.36 ^c
VFA (mM)	128.51±20.43	128.87±17.14	146.01±15.26
Microbial protein synthesis-(mg 10mL ⁻¹)	7.85±0.97	9.64±0.67	9.66±3.20

^a Notes are referenced using alpha superscripts show significantly different (P < 0.05)

^b P: NSM combined with RAC pollard

^c R: NSM combined with RAC rice bran

^d C: NSM combined with RAC corn

NSM combination with different RAC derived from pollard, rice bran, and corn showed that differences in RAC had a significant effect on NH₃ production. The highest NH₃ concentration was found in the rations with RAC pollard and lowest NH₃ concentration in rations with RAC derived from corn. NH₃ describes the degradation products of feed protein and or NPN, so that excessive amounts show inefficiency. The optimum amount of ammonia in the rumen is 6-21 mM [20]. In this study, the amount of ammonia is below the optimum limit which can indicate two things, namely the lack of ammonia or the amount of ammonia used by the rumen microbes to form MPS, so that the accumulation of ammonia in the rumen is reduced. The difference in RAC in rations containing NSM did not have significant effect on pH.

4. Conclusion

The use of NSM as much as 20% in rations combined with carbohydrate sources derived from corn can optimize the use of NSM as a source of feed protein in ruminants by encouraging the production of microbial protein synthesis that were useful for ruminants.

5. Acknowledgment

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