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Effect of Different Ratio Omega-3 and Omega-6 in Total Mix Ration on Productive Performance, Blood Metabolites and Estrous Characteristic of Ewes

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ABSTRACT

This study was conducted to compare the effect of different ratio of omega-3:omega-6 in total mix ration to blood metabolites and reproductive performance of ewes. A total of 25 young ewes were randomly assigned to five experimental groups and five replications : Ro (without omega-3:omega-6), R1 (omega-3:omega-6 1:8), R2 (omega-3:omega-6 1:6), R3 (omega-3:omega-6 1:3), R4 (omega-3:omega-6 1:2). The parameters measured were feed intake, productive performance, blood glucose and cholesterol pre-mating period. Parameters of characteristic estrous measured were onset of estrous, length of estrous and estrous response. The result showed that ratio of omega-3 and omega-6 1:2 have a greatest daily weight gain and feed efficiency. Ratio of omega-3 and omega-6 1:2 have blood glucose lowest, but highest blood cholesterol. Addition of omega-3 cause to delays onset of estrous. The conclusion is greatest length of estrous, estrous response and pregnacy rate on ratio of omega-3 and omega-6 1:2 in ration.

Keywords : Local ewes, Omega-3, Omega-6, Production performance, Total mix ration

Introduction

The increase in Indonesia's local sheep population depends on the success of the reproductive cycle. Intake of ewes such as carbohydrates, fats and proteins plays an important role in the success of the reproductive cycle. Nutrition is an important factor that has a lot of influence in the reproductive phase of livestock such as estrous and ovulation (Somchit *et al*., 2007). Less energy intake in a long time can delay puberty, disrupt the estrous cycle and prolong the postpartum anestrous period (Khotijah *et al*., 2014). Studies aimed at improving the reproductive performance of the ewes through nutritional manipulation have been carried out. One of them is by adding fatty acids in the ration. Fatty acids are not only needed as energy sources but also function as precursors for the formation of hormones that play a role in the reproductive cycle of livestock. Fatty acids will stimulate follicular development and steroid hormone production (Leroy *et al*., 2013) and improve fertility (Cerri *et al*., 2009).

Fatty acids in rations can have a positive impact on livestock reproductive performance. The fatty acids used in this study are omega-3 and omega-6 unsaturated fatty acids. Essential fatty acids in cow, especially linoleic and linolenic acid can increase the number and size of ovulated follicles, increase plasma progesterone and reduce the secretion of prostaglandin metabolites so that the life force of the corpus luteum increases and fertility can be improved (Staples *et al*., 1998). Khotijah *et al*. (2014) stated that the addition of omega-6 from sunflower seed oil can increase ovulation, embryo resistance, rate of birth, number of births of twins and number of births of male children in Garut sheep. Unsaturated fatty acids such as omega-3 and omega-6 can influence several factors related to the formation and metabolism of reproductive hormones such as steroid hormones, progesterone and estrogen (Gulliver *et al*., 2012). The formation of prostaglandin hormones is also influenced by omega-3 and omega-6 fatty acids. Unsaturated omega-6 fatty acids are precursors to the formation of the PGF2α hormone. Omega-6 in the body of livestock will be converted to arachidonic acid. Arachidonic acid is used by the body to form the PGF2α type prostaglandin hormone (Gulliver *et al*., 2012), while omega-3 fatty acids are precursors to the formation of the PGE3α hormone. Both of these hormones have

different functions in the livestock estrous cycle which affect the success of livestock reproduction.

The fatty acid profile in feed can be used as a way to improve livestock reproduction (Khotijah *et al*., 2014). Unsaturated fatty acids omega-3 and omega-6 cannot be synthesized in the body so they need to be supplied from rations. Source of omega-6 can be obtained from soybean oil, safflower oil and sunflower seed oil, while for sources of vegetable omega-3 is flaxseed oil . This study uses two types of oil, namely sunflower seed oil and flaxseed. Abayasekara and Wathes (1999) say the balance of omega-6 and omega-3 plays an important role in some aspects of livestock health, production and reproduction. The right balance between omega-3 and omega-6 will be studied and their effects on production performance, blood metabolites and estrous characteristics will be examined.

Materials and Methods

The livestock used were 25 local ewes from 5-6 months old and 19.07 ± 1.52 kg which were divided into five treatments and five replications. This study uses individual cages equipped with food and drinking water. The ration is given in the form of complete mixed ration with the composition of feed ingredients is Brachiaria humidicola grass, stacks, coconut oil cake, sunflower seed oil cake, flaxseed oil as a source of omega-3, sunflower seed oil as a source of omega-6, minerals and vitamin. The ration was prepared with 12% crude protein content and 70% total digestible nutrient (TDN). The ration is arranged isoenergy and isoprotein. Composition of the treatment ration is presented in Table 1.

Feeding, measuring Feed consumption and body weight

The adaptation period of the ration was carried out for two weeks. Feeding is given 45 days before the mating period up to two months after giving birth. The ration was given twice a day at 7:00 a.m. and 4:00 p.m., while drinking water was provided ad libitum. Feed consumption is calculated from the difference in the amount of feed given with the remaining feed. Measurement of feed consumption is carried out every day during the maintenance period. Weighing measurements are carried out every two weeks during the maintenance period.

Estrous synchronization and observation of the characteristics of estrous

Estrous synchronization was carried out twice with an interval of 11 days, ie injecting Prostaglandin (PGF2α) Luteolysis® hormone preparations with a dose of 1.0 mL head^{-1} intramuscularly (Nasirin *et al*., 2014). Estrous synchronization is done twice to make sure the corpus luteum has completely shed. The process of estrous synchronization was followed by observations of the characteristics of estrous namely estrous onset, estrous response and length estrous. The sign of estrous is when the animal does not resist when it is ridden (standing heat). Onset of estrous is determined from hours after injection until estrous occurs, whereas estrous duration is determined from the first hour of estrous until estrous ends. Estrous response is obtained from the number of estrous sheep compared to the number of mated ewe in one group.

Marriage period by combining female and male sheep with a ratio of 5: 1 within seven days after rationing of the treatment for 45 days. The marriage process is only carried out in one estrous cycle. Observation on the characteristics of estruus starts at 08.00 to 17.00. The characteristic parameters observed were the initial appearance of estrous (estrous onset) by recording the time after the second injection until the first estrous symptoms appeared. Estrous

Table 1. Composition of feed ingredients and nutrient of ration in dry matter

Feed ingredients	Treatments[1]				
	R0 (%)	R1 (%)	R2 (%)	R3 (%)	R4 (%)
Brachiaria h.	30	30	30	30	30
Stacks	22,75	23,67	23,60	23,38	23,14
Coconut oilcake	34,30	34,30	34,30	34,30	34,30
SS oilcake*	8,40	7,00	7,00	7,00	7,00
SS oil*	0,00	0,43	0,41	0,35	0,25
CaCO3	0,70	0,70	0,70	0,70	0,70
Salt	0,35	0,35	0,35	0,35	0,35
Premix	0,70	0,70	0,70	0,70	0,70
Soybeans	2,80	2,80	2,80	2,80	2,80
Flaxseed Oil	0,00	0,05	0,14	0,42	0,77
Food substances (*nutrient*)					
TDN	69,85	70,34	70,33	70,30	70,26
Crude Fiber	21,54	21,02	21,00	21,00	21,00
Crude protein	11,74	11,47	11,47	11,46	11,46
Crude fat (*Ether extract*)	4,52	4,81	4,86	5,03	5,22
Calsium	0,52	0,51	0,51	0,51	0,51
Phospor	0,37	0,36	0,36	0,36	0,36
Omega-3**	0,09	0,12	0,17	0,33	0,52
Omega-6**	0,74	1,03	1,03	1,03	1,03
Ω-3: Ω -6	1:8	1:8	1:6	1:3	1:2

[1] R0 : without omega-3:omega-6, R1 : omega-3:omega-6 1:8, R2 : omega-3:omega-6 1:6, R3 : omega-3:omega-6 1:3, R4 : omega-3:omega-6 1:2.* SS oilcake: Sunflower Seed oilcake, SS oil: Sunflower Seed oil. ** Results of IPB integrated chemical laboratory analysis.

duration is calculated as the time period of the onset of the first estrous until the end of estrous observed during seven days of mating (Hafez and Hafez, 2000). The estrous response was calculated by dividing the number of estrous ewes with the number of ewes that were injected with PGF2α multiplied by 100 percent (Toelihere, 1981).

Collection of pre-mating parent blood samples

Blood samples are used to analyze glucose and cholesterol levels during the pre-mating period. Blood samples in the pre-mating period were taken on day 45 before mating to determine the effect of the ration on the concentration of glucose and cholesterol in the blood needed during the marriage process. Glucose is needed as an energy source while cholesterol is needed as a precursor to the formation of reproductive hormones. Blood is taken from the jugular vein using a 5 mL sterile spoit, then a blood sample is inserted in a tube containing anticoagulants in the form of EDTA. Blood samples were then centrifuged at 3,000 rpm for 15 minutes, until plasma was obtained.

Analysis of glucose and plasma cholesterol

The levels of glucose and cholesterol were determined using enzymatic colorimetric test (Glucose kit® Cat. No. 112 101, reg. No. AKL 20,101,803,460 and Cat Cholesterol kit® No. 101 592, reg No. AKL 10,101,803,466). The equipment used is a spectrophotometer, micropipette and test tube. In the analysis phase, first mixing 10□L of blood plasma samples with 1,000□L of reagent kit in a test tube. Preparation of blank solution was done by mixing 10□L of distilled water with 1,000□L of reagent kit in a separate test tube, then each was homogenized using vortex and incubated for 10 minutes at 25^{o}C, then the wavelength was read using Genesys® 10S UV-Vis spectrophotometer with a wavelength of 500nm. Readable numbers are the absorbance values of each sample and standard. Glucose levels are calculated using the formula:

$$\text{Glukosa (mg/dL)} = \frac{\text{sample absorbance}}{\text{standard absorbance}} \times 100$$

The cholesterol level of the sample is calculated using the formula:

$$\text{Cholesterol (mg/dL)} = \frac{\text{sample absorbance}}{\text{standard absorbance}} \times 200$$

Experimental design and data analysis

The experimental design used was a completely randomized design with five treatments and five replications. The treatment given is a type of ration with a different balance of omega-3 and omega-6, namely: R0 = ration without supplementation of omega-3 and omega-6, R1 = ration with a balance of omega-3 and omega-6 1: 8, R2 = ration with a balance of omega-3 and omega-6 1: 6, R3 = ration with a balance of omega-3 and omega-6 1: 3, and R4 = ration with a balance of omega-3 and omega-6 1: 2.

Result and Discussion

Feed intake and performance of pre-mating sheep

Dry matter consumption was not affected by treatment (P> 0.05). Adding 5% fat in the ration has not reduced the consumption of dry matter. Khotijah *et al*. (2014) stated that adding fat up to 6% did not reduce the consumption of dry matter. Dry matter consumption in the pre-mating period ranged from 478.58 - 515.97 g / head / day or 2.11 - 2.53% of body weight (Table 2).

Dry matter consumption in this study was lower than Kearl (1982) which stated that dry material consumption for sheep with body weight of 16-18 kg was 3.1 - 3.2%, but this did not affect sheep production performance. The different levels of omega-3 and omega-6 do not affect the consumption of dry matter but the balance of omega-3 and omega-6 1: 2 has the highest consumption of dry matter, namely 515.97 g / head / day. This high consumption of dry matter is expected to support better reproductive performance of the ewes.

The different levels of omega-2 and omega-6 did not affect consumption of crude fat (P> 0.05) (Table 2). This is because the crude fat content contained in the ration is relatively the

Table 2. Nutrients consumption and productive performance of ewes pre-mating

Variables	Perlakuan (treatments)[1]				
	R0	R1	R2	R3	R4
Dry matters (g head^{-1} day^{-1})	501,44±85,61	481,54±64,88	478,58±64,69	504,91±63,20	515,97±103,24
Crude fat (*Ether extract*) (g head^{-1} day^{-1})	22,97±3,59	23,14±3,12	23,26±3,14	25,39±3,18	26,94±5,39
Total digestible nutrient (g head^{-1} day^{-1})	350,80±59,26	338,73±45,64	336,59±45,50	354,94±44,43	362,51±72,53
ω-3 (g head^{-1} day^{-1})	0,47±0,08^{a}	0,58±0,08^{a}	0,82±0,11^{a}	1,65±0,21^{b}	2,70±0,54^{b}
ω-6 (g head^{-1} day^{-1})	3,70±0,63^{a}	4,94±0,48^{a}	4,91±0,66^{b}	5,19±0,65^{b}	5,26±1,05^{b}
ω-3:ω-6	1:8,0	1:8,5	1:6,0	1:3,1	1:2,0
Dry matters consumption (%DM)	2,32±0,30	2,53±0,20	2,11±0,49	2,39±0,23	2,37±0,36
Daily gain (g head^{-1} day^{-1})	49,24±7,98^{a}	26,52±5,25^{b}	50,00±2,27^{a}	51,52±5,72^{a}	59,09±18,18^{a}
Efficiency of ration (%)	9,52±1,74^{ab}	5,47±1,68^{b}	10,42±1,83^{ab}	10,90±1,44^{a}	11,95±4,88^{a}

[1]R0 : without omega-3:omega-6, R1 : omega-3:omega-6 1:8, R2 : omega-3:omega-6 1:6, R3 : omega-3:omega-6 1:3, R4 : omega-3:omega-6 1:2. *different superscripts at the row indicate significance difference P<0.05),

same, namely 4, 52 - 5.22% (Table 1). Crude fat consumption was not significantly different (P>0.05) but the addition of unsaturated fatty acids in the ration showed an increase in fat consumption compared to R0 treatment (without supplementation of omega-3 and omega-6) although the ration fat content was relatively the same. Increasing fat consumption is expected to increase fatty acids in the body as a precursor to the formation of reproductive hormones. The highest consumption of crude fat is found in the omega-3 and omega-6 1: 2 balance treatment, which is 26.94 g / head / day. This is consistent with higher dry matter consumption in the same treatment. Consumption of total digestible nutrients (TDN) is relatively the same in all omega-3 and omega-6 counts. TDN consumption (Table 2) is still within the standard range of Kearl (1982) which states that TDN consumption for sheep with body weights of 16-18 kg is 304-332 g / head / day.

The addition of omega-3 and omega-6 with different balances greatly affects the consumption of omega-3 and omega-6 (P <0.05). Omega-3 consumption increases with the addition of omega-3s in the ration. The treatment of R4 had higher consumption of omega-3 and omega-6 than other treatments, because the consumption of dry matter in the treatment was also higher than other treatments (Table 2). Consumption of high omega-3 and omega-6 is expected to increase the chances of omega-3s and omega-6s being deposited more in the body so that they can support reproductive performance through the provision of energy and an increase in hormone precursors.

Daily body weight gain and feed efficiency are influenced by different omega-3 and omega-6 balance (P <0.05). The highest daily body weight gain was in the R4 treatment, which was 59.09 g / head / day, while the lowest daily body weight gain was in the R1 treatment, which was 26.52 g / head / day. This shows that the higher the unsaturated fatty acids in the ration are not in harmony with the increase in daily body weight gain. Daily body weight gain is more determined by the consumption of dry ingredients. High dry matter consumption in R4 treatment can be converted into growth in the form of daily body weight gain. This is also reflected in the value of feed efficiency. The efficiency of rations in the R4 treatment was also higher than other treatments. The ration efficiency value in this study is in line with Hersade's (2015) study which states that the value of feed efficiency in local sheep given 4% sunflower seed oil can reach 9%.

Blood metabolites in pre-mating period

Glucose and cholesterol levels are presented in Table 3. Pre-mating glucose levels are influenced by treatment. Glucose levels in R4 (1: 2) treatment were lower than other treatments, this value was also lower than the study of Khotijah *et al*. (2014) for sheep fed with sunflower seed oil that is 47.82 - 79.67 mg / dL. Cholesterol levels in R4 treatment were significantly higher than other treatments (P <0.05). The conversion of glucose to pyruvic acid is converted to acetyl-CoA which is needed in cholesterol synthesis through the HMG-CoA pathway. This process indicates the cause of low blood glucose accompanied by high cholesterol levels.

Higher cholesterol levels are found in the omega-3: omega-6 1: 2.0 balance treatment (Table 3). In this study the source of omega-3 uses falxseed oil while for the source of omega-6 uses sunflower seed oil. The addition of fat in the form of omega-3 from flaxseed has a higher plasma cholesterol level compared to cholesterol levels with the addition of sunflower seed oil (Akbarinejad *et al*., 2012). Increasing cholesterol levels in the blood can be used as precursors for steroid hormones such as estrogen and progesterone.

Characteristics of estrous and percentage of pregnancy

The estrous cycle is included in the critical phase of the reproductive cycle. The success of the estrous cycle increases the percentage of pregnancy. The estrous cycle occurs when the corpus luteum decays by the hormone prostaglandin type PGF2α. Estrous characteristics parameters consist of estrous onset, estrous length and estrous response are presented in Table 4.

The different levels of omega-3 and omega-6 in the ration affected the onset of estrous (P<0.05). The omega-3: omega-6 1: 2 balance has a slower onset of estrous than other counterparts. High omega-3 content in this treatment is indicated as a cause of delay in estrous onset. In line with the study of Nieto *et al*. (2015) which states the addition of omega-3 from fish oil can slow the onset of estrous in sheep.

Delay of estrous onset as a result of a decrease in arachidonic acid which is a precursor for the formation of the prostaglandin hormone (PGF2α) (Gulliver *et al.*, 2012). The use of the same enzyme between omega-3 elongase to eicosapentaenoic fatty acids and omega-6 elongase to become arachidonic acid is the cause of the decrease in the precursor forming hormone

Table 3. Level of glucose and cholesterol plasma during pre-mating period

Variables	Treatments[1]				
	R0	R1	R2	R3	R4
	(mg/dL)				
Glucose	46.15±5.52^{ab}	55.50±6.49^{a}	53.71±3.19^{a}	51.35±14.85^{ab}	39.71±5.63^{b}
Cholesterol	79.79±18.04^{ab}	79.09±4.33^{ab}	55.71±3.52^{b}	67.82±11.46^{ab}	86.94±2.74^{a}

[1]R0 : without omega-3:omega-6, R1 : omega-3:omega-6 1:8, R2 : omega-3:omega-6 1:6, R3 : omega-3:omega-6 1:3, R4 : omega-3:omega-6 1:2. * different superscripts at the row indicate significance difference P<0.05

Table 4. Characteristic of estrous and pregnancy rate

Variables	Treatments[1]				
	R0	R1	R2	R3	R4
Onset of estrous (hour)	21.14±0.19[a]	46.94±26.03[a]	53.64±12.70[a]	126.95±30.11[b]	132.61±36.75[b]
Length of estrous (hour)	47.51±20.10	54.74±38.38	30.35±13.94	24.63±2.52	27.94±20.49
Response of estrous (%)	80 (n=4)	80 (n=4)	80 (n=4)	60 (n=3)	100 (n=5)
pregnancy rate (%)	80 (n=4)	80 (n=4)	80 (n=4)	60 (n=3)	100 (n=5)

[1]R0 : without omega-3:omega-6, R1 : omega-3:omega-6 1:8, R2 : omega-3:omega-6 1:6, R3 : omega-3:omega-6 1:3, R4 : omega-3:omega-6 1:2. * different superscripts at the row indicate significance difference P<0.05)

PGF2α. Unsaturated fatty acids α-linolenic (omega-3) will experience elongase with the help of the elongase Δ6-desaturase enzyme to become eicosatetranoic fatty acids which then results in the addition of double bonds with the help of the enzyme Δ5-desaturase to eicosapentaenoic fatty acid (EPA) (Clayton *et al*. , 2007; Gulliver *et al*., 2012). Eicosapentaenoic fatty acid (EPA) is a precursor for the formation of the PGE3α type prostaglandin hormone (Gulliver *et al*., 2012). This type of hormone works in proportion to the PGF2α hormone, resulting in a delay in the onset of estrous.

Onset of estrous was strongly influenced by treatment (P <0.05; Table 4). Based on the results of this study indicate that there are differences in the effect of saturated fatty acids and unsaturated fatty acids in the ration. Treatment of R0 or without the addition of omega-3 and omega-6 unsaturated fatty acids experienced estrous onset faster than other treatments (Table 4). This is indicated as the influence of the production of the prostaglandin hormone mentioned above. It also showed that unsaturated fatty acids in ruminant bodies had an influence on reproductive hormone production although long chain unsaturated fatty acids (PUFA) experienced rumen biohidrogenation of 86% for linoleic and 82% for linoleic (Jenskins and Bridges, 2007). Unsaturated fatty acids omega-3 and omega-6 have an influence on the estrous phase indicated because the sources of omega-3 and omega-6 used are from plant sources. Murphy *et al*. (1987) stated that some oil sources from plants are more resistant to rumen microbes than others.

The duration of estrous is not affected by the different balance of omega-3 and omega-6. The duration of estrous in this study ranged between 24.63 - 54.74 hours. This result is greater than Pineda and Dooley (2003) which states the estrous length in sheep is 20-36 hours. The omega-3 and omega-6 1: 8 balance has a longer estrous duration indicated by the addition of omega-6 without the addition of omega-3. The addition of omega-6 as a precursor to the PGF2α hormone prolongs estrous. In harmony with Khotijah *et al*. (2014) which states the addition of omega-6 in the ration can prolong the length of estrous. Estrous that lasts longer increases the chances of marriage and pregnancy.

The estrous response in this study ranges from 80-100%. The treatment of R4 with the balance of omega-3 and omega-6 1: 2 has the best estorus response. This value is higher than the study of Khotijah *et al.* (2014) that is 50-75% in ewes given sunflower seed oil. Percentage of estrous response in R4 treatment showed an improvement in the reproductive system by adding omega-3 and omega-6 1: 2 unsaturated fatty acids. This was indicated because cholesterol levels in the R4 treatment were higher than other treatments (Table 3). High cholesterol is used as a precursor to the formation of reproductive hormones, namely estrogen. One of the main functions of estrogen is to stimulate lust (estrous) (Siregar, 2009). Pregnancy percentage in this study is 60-100% (Table 4). Akbarinejad *et al.* (2012) with the addition of sunflower seed oil resulted in a pregnancy percentage of 73.91%, whereas with the addition of flaxseed oil (omega-3) resulted in a pregnancy percentage of 59.57%. This shows that the combination of omega-3 from flaxseed oil and omega-6 from sunflower seed oil with the right balance can increase the percentage of pregnancy.

Conclusions

The omega-3 and omega-6 1: 2 balance has the best daily body weight gain and ration efficiency. The omega-3 and omega-6 1: 2 balance has low glucose levels but the highest cholesterol level. Addition of omega-3 compresses the onset of estrous. Duration of estrous, estrous response and percentage of pregnancy is best with a balance of omega-3 and omega-6 1: 2 in the ration. Sunflower seed oil and flaxseed can be used to improve livestock reproductive performance.

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