

THE INCREASE OF OMEGA-3 BY FEEDING BALI CATTLE
WITH SUPPLEMENTATION LEMURU FISH OIL
IN MOLAMIX CONCENTRATE

N.L.P. Sriyani, S. Putra, IK., Saka dan I.B. Gaga Partama

Doctoral Study Program of Animal Science Post Graduate Udayana University

Jl . PB Sudirman Denpasar

E-mail : sriyaninlp@yahoo.co.id

085857261149

ABSTRACT

This study was carried out to increase the content of Omega-3 (EPA and DHA) on beef by feeding bali cattle with supplemented lemuru fish oil in Molamix concentration. A Randomized Complete Block Design was applied consists of 3 treatment diets and 4 weight groups as replication. There were twelve Bali cattle used at range weigh of 248-277 kg. The cattle randomly allocated into 3 treatments fed with elephant grass + concentrate without fish oil supplementation as control (RKMBI₀); elephant grass + concentrate with lemuru fish oil supplementation 5% (RKMBI₅); and lemuru fish oil supplementation 10% (RKMBI₁₀). The study showed that lemuru fish oil supplementation decreased dry matter consumption (BK) in accordance with the increase of concentration energy content. Fish oil supplementation produces ALA in beef fat RKMBI₀ at 0.18%, RKMBI₅ at 0.20% and RKMBI₁₀ at 0.14 % which was not significantly different. While the EPA content in beef fat RKMBI₅ at 0.07% higher than the beef meat RKMBI₀ at 0.05%, and RKMBI₁₀ at 0.04 %. RKMBI₅ beef content of DHA. In contrary, 0.06 % was not found on RKMBI₀ and RKMBI₁₀ beef. The amount of omega-3 content in each 100 g of RKMBI₅ at 1.16 mg and RKMBI₁₀ at 1.75 mg significantly ($P < 0.05$) higher than RKMBI₀ at 0.60 mg. It can be concluded that fish oil supplementation in molamix lemuru concentrate decreased ration palatability which lead to decrease dry matter consumption. However, fish oil supplementation significantly increased the amount content of omega-3/100g on bali cattle.

Keywords : lemuru oil , meat of omega-3, bali catle, feed intake

INTRODUCTION

In general, the cattle fatty industry in Bali is conventional. Mainly, feeding cattle aims to improve weight body without paying attention to meat quality produced. Recently, most study of cattle fatty industry conducted with various concentrate on grass feed which has not been oriented to a higher quality of animal products, especially omega-3. Eventhough forage and concentrate utilized as ration material containing enough precursor of omega-3, but livestock as biological industry has no capability in producing an efficient omega-3 meat (*Eicosapentaenoic Acid* = EPA and *Dokosaheksaenoic Acid* = DHA).

The society whose conscious in a healthy life need cattle production, such as: milk, and meat containing omega-3, (EPA:C20:5 n-3 and DHA; C22:6-n3), especially for pregnant mother and infants as well as old age people. This is due to the important of micronutrient for their brain development related with intelligence and prevention of arteriosclerosis and also heart attack. Meanwhile, animal products and its processing products distributed in market are not promoting herbal omega-3, but through supplementation method. Thus, usage efficiency is relatively low mainly for the effectiveness to increase Indonesia's children ability so it effect less beneficial for consumers. In that consequence, strategy for diversification of omega-3 on animal feed through nutrition bio-manipulation for bali cattle is required.

Lemuru fish oil (*Sardinella longiceps*) is mostly derived from unsaturated fatty acids with long chains or PUFA (*Polyunsaturated Fatty Acid*) especially EPA 16.6% and DHA 12.79% (Yurleni, 2012) that can be used as food materials in formulating ration for beef. 75 mg/kg ZnSO₄ was mixed with 1.5% lemuru fish oil in ration of FH cattle can increase Zn (22 vs 30 mg/day) absorption compared to control ration in relation with the increase of daily weight body (1.7 vs 0.49 kg/head) as well as EPA and DHA incorporated with meat fosfolipida, respectively 2.71% and 0.88% (Hartati, 1988)

EPA and DHA fatty acids are unsaturated fatty acids with long chain of carbon atoms (C₂₀) and (C:22). The study stated that unsaturated fatty acid in long chain is difficult in rumen hydrogenation, so stock of unsaturated fatty acids can be enhanced (Ashes et al., 1992). The increase supply of unsaturated fatty acids especially EPA and DHA are expected to be stored in the intestine or muscle meat (Cristie, citation Widiyanto 2008). Therefore, fish oil supplementation of lemuru into Bali cattle ration expected to enrich the content of EPA and DHA meat.

MATERIALS AND METHODS

Cattle and Feed

This study was used 12 male bali cattle with the initial weight of 262.75 ± 11.27 kg. These were obtained and purchased from the Beringkit Animal Market, at Badung regency. Those cattle were fed with a basic feed such as elephant grass and reinforcement grass in the form of Molamix concentrate as much as 3 kg during the

fattening period. Feed supplement used for concentrate as of: rice bran, corn bran, pollard, coconut meal, yellow corn, molasis, mixed mineral salt, urea and oil lemur.

Research Design

This study was using a randomized treatment A Complete Design Group (A Randomized Complete Block Design) consists of : 3 and 4 diets weight group as a group so totally 12 cattle used. Bali male cattle with an average body weight: 277 ± 4.3 kg in group I; 265.67 ± 4.7 kg in group II; 260.33 ± 1.5 kg in group III; and 248 ± 1.7 kg in group IV. The cattle were randomly allocated into 3 treatments fed with the control treatment and elephant grass molamix concentrate without fish oil (RKMBI₀), elephant grass and molamix concentrate of 5 % oil fish (RKMBI₅) and elephant grass and molamix concentrate of 10 % oil fish (RKMBI₁₀).

Elephant grass was fed in *ad libitum* and cut with chopper engine on the whole treatments. Molamix concentrate was given twice a day as much as 3 kg/day by mixing it with grass. Three treatments ration was conducted as below:

RKMBI₀ treatment : elephant grass + molamix concentrate without fish oil

RKMBI₅ treatment : elephant grass + molamix concentrate of 5 % oil fish

RKMBI₁₀ treatment : elephant grass + molamix concentrate of 10 % oil fish

The Composition and Feed Mixed Concentrate

Concentrate feed was composed based on the chemical composition from raw materials that has been determined previously through proximate analysis (AOAC,

1990). Further, the mixed of raw materials was manually emphasized on homogeneity mixed results obtained. The mixed ration treatment, especially elephant grass with concentrate was conducted by observing its acceptability based on dried matter standard consumed (% DMI from cattle body weight; Kearn, 1982). This nutrient content of ration formula (Table 1) was expected to able to fulfill the beef standard weighing 300 kg with additional weight 0.75 kg per cattle per day, namely in each metabolizable energy (ME) = 15.80 M Cal and protein = 0.753 kg.

Table 1
Ration nutrient content in treatment of elephant grass and
Molamix concentrate of fish oil

Nutrient rations	Molamix concentration of fish oil		
	RKMBI ₀	RKMBI ₅	RKMBI ₁₀
Dry matter	46,76	47,97	48,30
Organic Materials	86,54	86,88	87,02
Energy (kcal/kg)	4128,20	4174,69	4260,40
Crude protein	11,50	11,31	11,02
Crude fat	4,58	5,59	6,95
NDF	67,48	66,47	66,77
ADF	44,69	44,39	43,58
Ash levels	13,46	13,12	12,97
Linolenat (%)	0,48	0,48	0,47
EPA (%)	-	0,24	0,47
DHA (%)	-	0,25	0,5

Location and Period of Research

In Vivo research was conducted at a garden owned by Yani Yuhani Panigoro, managed by PT. Sarana Bali Ventura, Denpasar. It was located at Banjar Asah Gobleg, Buleleng regency, 1250 – 1400 above sea level. The slaughtering was carried out at Temesi slaughtering house, Gianyar regency. This study was conducted for 8

(eight) months.

Dry mater intake and Omega-3

Dry matter intake of ration is dry matter of elephant grass and dry matter concentrate. In dry matter, the intake can be calculated by multiplying per kilogram of fresh elephant grass with dry matter composition; reducing the result of multiplication (remains of fresh elephant grass and feed). The intake of dry matter could also be calculated as follows:

Consumption of dry matter concentrate = (% dry matter concentrate x concentrate fed, kg dry matter of air) minus (% dry matter concentrate x remain of concentrate, kg dry matter of air). Then, the consumption of omega-3 (ALA, EPA and DHA) was calculated by multiplying level of each omega-3 with the amount of dry matter consumed per kg per cattle per day in each treatment.

Intramuscular fat (marbling)

The meat sample on *longissimus dorsi* muscle was extracted by hexane using Soxhlet method. Two samples were put on cotton with filter paper, rolled and inserted into the Soxhlet flask. The extraction was conducted for 6 hours with 150 ml of solvent fat. The extracted fat was dried in the oven at 100⁰C for 1 hour. Fat content can be calculated with the following equation:

$$\% \text{ fat content} = \frac{\text{weight of extracted fat}}{\text{weight sample}} \times 100\%$$

Omega-3 Content of Meat

The determination content or composition of fatty acids from each meat sample (*M. longissimus dorsi* muscle) has been extracted by using AOAC 1984 procedure. The analysis procedure was conducted with gas chromatography instrument (GC) of Shimadzu GC-9AM and Hewlett Packard (HP) 6890 series. The formula approach was as below:

$$C_x = \frac{A_x \cdot R \cdot C_s}{A_s}$$

Description:

C_x = component concentration x

C_s = standard concentration internal

A_x = area of peak component x

A_s = area of internal standard peak

R = response of detector to x component relative to standard

Statistics Analysis

The data obtained in this study was analyzed by using variance analysis, SPSS 16 series program in 2008. In case tangible result was found, then further analysis of Duncan test, Multiple Range Test at 5% significant limit conducted (Steel and Torrie, 1989).

RESULTS AND DISCUSSIONS

Dry Matter Consumption and Omega-3

The consumption of dry matter on cattle fed RKMBI₂ and RKMBI₁₀ treatments were 14.7% and 21.9% significantly lower ($P<0.05$) compared to control treatment (RKMBI₀ in Table 2). Moreover, dry matter RKMBI₁₀ fed to cattle was 8.42% significantly lower ($P<0.05$) than RKMBI₅. This is due to higher oil supplementation of fish on *Molamix* concentrate (5 – 10%) can increase the content of ration energy (Table 1), so dry matter consumed was relatively low. Based on Putra (1992; 1999), ration energy correlates negatively with level of dry matter ration that higher ration energy could give effect to lower level of dry matter consumption.

According to Putra (1999), in physiologis the level of dry matter consumption and ration of organic material basically used to fulfill the energy needs of cattle. In case it has been fulfilled so nerve stimulated to stop consuming although its rumen capacity has not reach maximum or vice versa. Hartati (2008) also found that 3% oil fish supplementation on 100% ration of FH male cattle affected to decrease the level of dried and organic materials consumption.

Omega-3 consumption represented in linolenat acid consumption were EPA and DHA consumption (Table 2). Consumption of linolenat on RKMBI₀ was 23.5% significantly higher ($P<0.05$) compared to RKMBI₁₀ and 14.6% higher if compared with RKMBI₅ but differs significantly ($P>0.05$) in statistics. Meanwhile, the consumption of EPA on RKMBI₅ and RKMBI₁₀ was insignificantly differs. EPA and

DHA consumption on RKMBI₀ were not available due to control feed did not contain EPA and DHA. Omega-3 consumption trends in accordance with dry matter consumption on cattle research. This physiologic condition describes that dry matter ration consumption determine as the indicator of ration nutrient consumption. Putra (1992) also explained that dry matter ration consumption has a positive correlation with ration nutrient consumption.

Fat Content of Intra Muscular/Marbling

Intramuscular fat (*marbling*) on beef and RKMBI₅ and RKMBI₁₀ was significantly higher ($P < 0.05$) compared to control cattle. It showed that fish oil supplementation significantly effect the increase on intramuscular fat content. This is due to the fish oil supplementation led to increase fat digestibility. Fish oil added in the concentrate treatment will be the fat component of feed ration, external structure of particles. In that case, direct contact will be executed with rumen microbial and degradative enzymes so it could increase fat digestion.

Table. 2

The effect of Fish Oil Supplementation on Feed Consumption and Omega-3 content of intramuscular fat and content of Omega - 3 on Fatty Beef of Male Bali Cattle

Variabel	Treatment			SEM
	RKMBI ₀	RKMBI ₅	RKMBI ₁₀	
Dry Matter Consumption (kg)	6.86 ^a	5.85 ^b	5.36 ^c	0.32
ALA intake (g/e/h)	32.92 ^a	28.10 ^{ab}	25.19 ^b	7.78
EPA consumption (g/e/h)	-	14.05 ^a	13.40 ^a	0.97
DHA consumption(g/e/h)	-	27.51 ^a	26.80 ^a	1.93
Fat content				
Intramuscular / marbling	0.26 ^c	0.38 ^b	0.91 ^a	0.01
ALA content of meat (%)	0.18 ^a	0.20 ^a	0.14 ^a	0.03
EPA content of meat (%)	0.05 ^b	0.07 ^a	0.04 ^b	0.01
DHA content of meat (%)	nd	0.06	Nd	

Remarks:

RKMBI₀ = Ration control (elephant grass + molamix concentrate without fish oil)

RKMBI₅ = Elephant grass + 5 % concentrate oily fish molamix

RKMBI₁₀ = Elephant grass + 10 % concentrate molamix oily fish

Different superscripts in the same row are significantly different (P<0.05)

SEM = Standard Error of the Treatment Means

nd = non detection

The fat entered into rumens will directly transform as lipolisis and biohydrogenation process. Fish oil supplementation caused unsaturated fatty acid MUFA (*monounsaturated fatty acids*) with carbon chain under 20 (C.20) as of: oleat acid C18:1 and palmitoleat C16:1 on RKMBI₅ and RKMBI₁₀ treatment increase. The

content of PUFA mainly C:18n3, (Linolenic), C:20n3 (EPA) and C:22n3 (DHA) on treatment ration can also increase with the availability of fish oil supplementation.

Ashes *at al* (1992) stated that long link of unsaturated fatty acids, especially C:20 and C:22 carbon links are quiet difficult to be hydrogenised by rumen bacteria. The availability of unsaturated fatty acid passed from most of bio-hydrogen then being absorbed by the organ of post rumen digestion. Cristie (1979) explained that unsaturated fatty acids that passed from rumen bio-hydrogen will be quickly absorbed instead of saturated fatty acid since its hydrophilic characteristic. Similar result reported by Erwanto (1995) that additional fish oil in ration can increase raw fat digestion.

Omega-3 content on Fat Meat

In this study Linolenic acid content of meat taken from the muscle sample of *m.longissimus dorsi* was RKMBI₅ beef at 11.1% higher ($P > 0.05$) than control. In contrary, linolenic acid was RKMBI₁₀ beef at 22.2 % lower than control beef but did not differ significant ($P > 0.05$) in statistic. The results showed that fish oil supplementation in concentrate did not provide give significant effect for increase of linolenic fatty acids. Although linolenic fatty acid intake on cattle control was the highest, but it did not necessarily give a high yield on linolenic acid content of meat. Partially, linolenic fatty acids consumed in rumen hydrogenated and loosen hydrogenated as precursor used in the formation of EPA.

The availability of linolenic acid content in meat sample found that not all saturated fatty acids under long chain C;20 hydrogenated by rumen bacteria. In general, linolenic fatty acid C18:3 n-3 was hydrogenated by rumen bacterium of stearic acid C18:0. Jenkins (1993) stated that the level of hydrogenation of unsaturated fatty acids depend on the degree and amount in ration and frequency of administration. Furthermore he stated that hydrogenation of PUFA, such as: linoleic acid and oleic acid in the rumen estimated 60-90 %, whereas unsaturated fatty acid of C:20 length chain was slightly process hydrogenation in the rumen (Chilliard et al. 2000). Linolenic fatty acid C18:3 n3 is an essential fatty acid meant that tissues of animals unable to synthesize so it must be obtained from food (*oral supplementation*). In this study, Linolenic fatty acids C18:3 n3 on concentrate control was 0.48 % that might be derived from fatty acid as building blocks of concentrated coconut meal and yellow corn (Bressani, 1990).

The average content of EPA on RKMBI₅ beef was 40 % significantly higher than ($P<0.05$) RKMBI₀ cattle. In contrary, RKMBI₁₀ was 20 % lower compared to RKMBI₀ cattle ($P>0.05$). It can be illustrated that 5% fish oil supplementation concentrate significantly effect the increase of EPA content on meat. EPA fatty acids contained in meat ration reflect lipid metabolism ration in the rumen. Ruminant tissues did not synthesize PUFA, so concentration in tissues of body depend on the amount output of rumen.

The content of EPA control on beef caused by some potential components of concentrate feed as precursor. According Sardesi (1992), polyunsaturated fatty acid

(PUFA) in mammalian tissues derived from four precursors as of: palmitoleic acid, oleic acid, linoleic acid and linolenic acid. In addition, desaturation and elongation of four precursors form in long-chain fatty acids polyunsaturated (PUFA) based on requirement of mammalian body. Palmitoleic acid (C16:1, n-7) and oleic acid (C18:1, n-9) can be synthesized in endogenous, each of palmitic acid (C 16:0) and stearic acid (C18:0). Linoleic acid (C18: 2 n - 6) and linolenic acid (C18:3, n - 3) should obtained from feed and called as essential fatty acids (Lingga, 2008).

The main product of desaturation and elongation of linolenic acid are eicosapentaenoic acid (C 20:5, n-3) or EPA and docosahexaenoic (C22 6, n-3) or DHA (St. John, et al 1991) and Stryer (1996). The content of EPA in beef control derived from linolenic acid that passed from rumen hydrogenation then processed in desaturation and elongation to form EPA (Cunnane and Griffn, 2002). However, EPA content in RKMBI₅ beef was significantly higher (P<0.05) compared to control cattle. This is considered to be the cause of EPA content of meat on RKMBI₅ formed in two sources, namely the precursor in terms of linolenic acid from concentrate and fish oil supplementation that 4.7 % of EPA content was in fish oil. EPA content in beef RKMBI₁₀ slightly lower than control cattle allegedly as a decrease palatability of ration that depress feed intake and produced in the decrease in EPA content of meat.

Lipid escaped from biohydrogenase rumen, absorption coefficient range between 80% and 92% for SAFA for PUFA on conventional feed with low fat content of 2-3 % dry matter (Bauchart, 1993). Subsequent physiological processes that lipids will be distributed in blood plasma to form lipoproteins. The lipoprotein

distribute lipid from secreting organ, namely the intestine and the liver to peripheral tissues. Chemical composition and the rate of secretion of these lipoproteins are the main factors that controlled lipid utilization by network, which in turn affect the qualitative and quantitative characteristic of animal products such as meat (Mandell, et al., 1995). In this study, EPA content found lower on meat compared to the result of study of Yurleni (2012) who found that the swamp buffalo were supplemented in 4.5 % fish oil protected in concentrate of beef EPA content 0.22% while in PO cattle produced beef EPA content 0.18%.

The content of DHA was not found on RKMBI₀ and RKMBI₁₀ beef while 0.06 % found on DHA of RKMBI₅ beef. This is presumably caused by content of DHA in RKMBI₅ beef derived from fish oil and deposited in the tissues. DHA content of RKMBI₁₀beef was not found, probably as a result of low nutrient intake causing more fatty acids converted into energy supplies to the integrity and performance of cattle. It can also be seen from the highest amount of metabolized energy in RKMBI₁₀ cattle (Table 5.3). It is indicated that a lot of the energy used for activities has not been stored yet as DHA fatty acid. The content of DHA in control beef (RKMBI₀) was not also found. It was assumed that precursor linolenic acid contained in the control has not been able to concentrate on the elongation to form DHA.

Technically, omega-3 can only be used after the body is converted into EPA and DHA. In mammals there are generally many factors that hinder the performance of desaturase and elongase enzymes such as age, gender, excessive consumption of saturated fats, diabetics and hipertrigliserdemia. Changes in omega-3 ALA into EPA

and DHA were very limited in the elderly, because the decrease in the number of desaturation and elongase enzymes. Similarly, in fetus and infants at a certain age can not change the omega-3 ALA into EPA and DHA (Lingga, 2012).

Omega-3 Production in 100 g in the meat

The result showed that linolenic acid/mg in 100g beef produced 180.85 % of $RKMBI_{10}$ significantly higher with the control (Table 3). In addition, there was 42.55% insignificant increase on $RKMBI_5$ beef with control ($P>0.05$). 5% up to 10% increase level of fish oil supplementation gave 97.01% significant higher to the production of ALA in 100g beef ($P<0,05$). EPA production was in line with the production of ALA that produced EPA on 30.77% of $RKMBI_5$ not significantly higher than the control but 230 % higher on beef $RKMBI_{10}$ compared to control. The increase of fish oil supplementation also gave 152.94% significant increase on EPA content. In this study, omega-3 total production (ALA, EPA and DHA) on $RKMBI_5$ beef was 93% and $RKMBI_{10}$ was 191% significantly higher than control ($P<0,05$). Moreover, the increase level of fish oil supplementation also gave significant effect ($P<0.05$) to 50.86% increase of omega-3 production.

Table 3

The Effect of Fish Oil Supplementation on Omega-3 Production per 100 g of Bali Cattle Beef

Variable	Ration Treatment			SEM
	RKMBI ₀	RKMBI ₅	RKMBI ₁₀	
ALA (mg)	0.47 ^c	0.67 ^b	1.32 ^a	0.09
EPA (mg)	0.13 ^c	0.17 ^b	0.43 ^a	0.08
DHA (mg)		0.32		
Total Omega 3	0.60 ^c	1.16 ^b	1.75 ^a	0.02

Remarks:

RKMBI₀ = Ration control (elephant grass + *molamix* concentrate without fish oil)

RKMBI₅ = Elephant grass + 5% concentrate oily fish *molamix*

RKMBI₁₀ = Elephant grass + 10% concentrate oily fish *molamix*

Different superscript on the same row is significantly different (P<0,05)

SEM = *Standard Error of Treatment Means*

The increase production of ALA and EPA and total omega-3 in 100 g beef and RKMBI₅ and RKMBI₁₀ caused by intra muscular fat content (*marbling*) on RKMBI₅ and RKMBI₁₀ increased significantly (Table 2). The increase of intramuscular fat content due to the increase of fat consumption and digestibility in RKMBI₅ and RKMBI₁₀ cattle. Fatty acids are the building blocks of virtually all components of lipids or fats (Lehninger, 1982), thus the increase of fat content of meat will be followed by the increase of fatty acid content both ALA, EPA and DHA.

This is similar to Ponnampalan et al. (2001), he stated that the type of fat in the domestication of cattle feed can affect the composition of the total fatty acids and neutral fat in the muscle tissue. Saturated fatty acids when given beyond the need will be deposited in fat tissue as triglycerides reserves, whereas PUFA mainly n-3 deposited largely in fospolipid structure.

The production of ALA, EPA, DHA and total omega-3 on RKMBI₀, RKMBI₅ and RKMBI₁₀ beef illustrated in the following graphic (Figure 5). Production of ALA, EPA, and total omega-3 experienced an upward trend in the treatment given fish oil supplementation in concentrates. However, DHA is only produced by beef RKMBI₅.

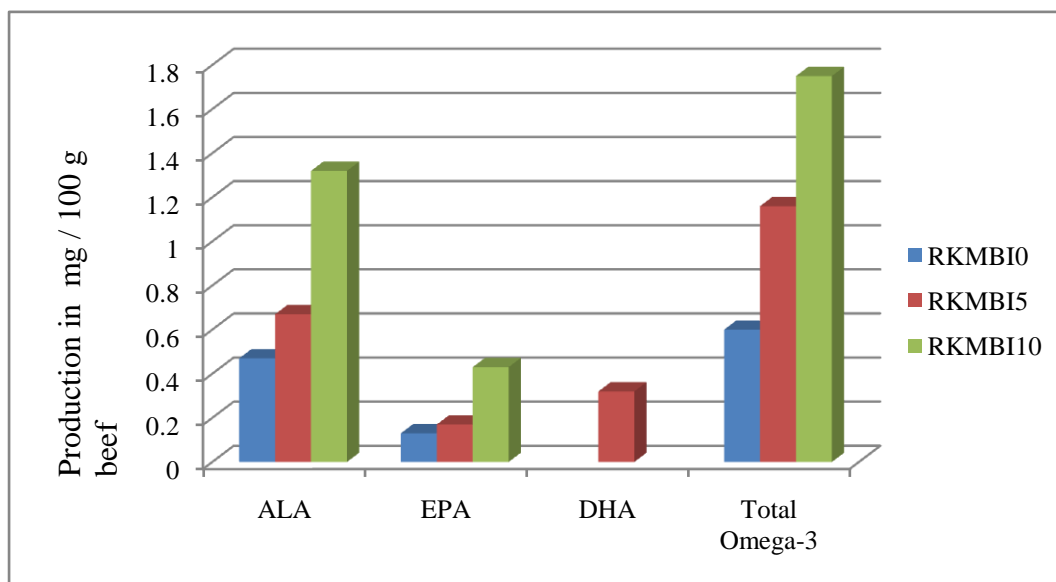


Figure 1. Production of ALA, EPA, DHA and total omega-3 of meats during research

Omega-3 is an essential fat that has benefit in all parts of our body. Performance of the omega-3 include the circulatory system and management glucose (Da Young, et al, 2007), brain health (Van de rest et al , 2008), and immune system. Omega-3 promotes production of eicisanoid that is useful to resolve inflammation, thin the blood, inhibit cell mutations, assist the digestive system, and regulate blood pressure (Luna, J et al, 2011). DHA for fetus is needed for the brain while EPA used to function brain memory in the future. Fetal and neonatal of certain age can not change omega-3 ALA into EPA and DHA so they receive from the intake of omega-3 from their mother in terms of EPA and DHA (Jansens, 2006).

In general, omega-3 sources are obtained from fish. It lives in a cold water temperature of sea and has higher omega-3 content compared to tropical fish (Mozafarian et al, 2006). For example, sardines fish in Japan's sea contain 37mg/100 g of omega-3. However, fish in tropical water such as in Indonesia generally contain 0.1-0.5 mg/100g of omega-3 and only few of Indonesia's local fish contain 10-11 mg/100 g of omega (Lingga, 2012).

CONCLUSION

1. Lemuru fish oil supplementation in molamix concentrate at the level of 5% and 10% can increase intramuscular fat content (*marbling*). Lemuru fish oil supplementation increased the content of EPA and DHA in fatty meats and increase the production of EPA and DHA in 100g Balinese beef.

2. The increase level of lemuru fish oil supplementation in molamix concentrate impact in quantitative on increasing production of EPA and DHA in 100 g of meat. However, in this study an ideal level of fish oil supplementation is at the level of 5 % which produce three types of omega-3 namely ALA, EPA and DHA.

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