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Blood Malondialdehyde, Reproductive, and Lactation Performances of Ewes Fed High PUFA Rations Supplemented with Different Antioxidant Sources

D. M. Fassah[a]*, L. Khotijah[a], A. Atabany[b], R. R. Mahyardiani[a], R. Puspadini[a], & A. Y. Putra[a]
[a]Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University
[b]Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University
Jalan Agatis, Kampus IPB Darmaga, Bogor 16680, Indonesia
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ABSTRACT

The aims of this study were to evaluate the effect of vitamin E (vit E) and black tea extract (BTE) as antioxidant sources in high poly-unsaturated fatty acid (PUFA) rations on blood malondialdehyde (MDA) concentrations and the performance of reproduction and lactation of Garut ewes. Twelve ewes on late pregnancy periods were divided into completely randomized design (CRD) with 3 treatments and 4 replicates. The treatments were control: basal diet without antioxidant, vit E: basal diet supplemented with vit E, and BTE: basal diet supplemented with BTE. The results showed that vit E and BTE supplementation did not affect blood MDA concentration of ewes on late pregnancy, lactation periods, and weaning periods. Antioxidant sources supplementation had no effect on ewe's nutrient intake, pre-lambing live weight change (LWC), and post-lambing average daily gain (ADG). Vit E supplementation had decreased the milk production, but both of antioxidant sources give positive effect on the milk composition. Nevertheless, the supplementation of vit E and BTE increased the twin type of birth. The vit E supplementation resulted higher lambing rate than BTE, although it had the highest mortality rate of twin lamb. BTE also had better lamb weaning weight than vit E. BTE and vit E gave similar results in ewes productivity on lactation period. In conclusion, this study confirmed that BTE was more effective used as antioxidant source than vit E to prevent the oxidative reaction of PUFA. Vit E supplementation on high PUFA ration reduced ewes milk production but it had similar reproduction performance with BTE.

Key words: antioxidant, black tea extract, ewes, lactation, reproduction, vitamin E

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pengaruh penambahan vitamin E (Vit E) dan ekstrak teh hitam (BTE) sebagai sumber antioksidan dalam ransum kaya *poly-unsaturated fatty acid* (PUFA) terhadap kadar malondialdehida (MDA) darah, performa reproduksi dan laktasi induk domba Garut. Penelitian ini menggunakan rancangan acak lengkap pola searah dengan 3 perlakuan dan 4 ulangan. Perlakuan yang diberikan adalah kontrol: ransum basal tanpa antioksidan, Vit E: ransum basal disuplementasi Vit E, dan BTE: ransum basal disuplementasi BTE. Hasil penelitian menunjukkan bahwa suplementasi Vit E dan BTE tidak mempengaruhi kadar MDA darah induk domba pada akhir kebuntingan, periode laktasi, dan pada saat periode sapih. Sumber antioksidan tidak berpengaruh terhadap konsumsi pakan, *pre-lambing LWC* dan *post-lambing* ADG induk domba. Suplementasi vitamin E menurunkan produksi susu, namun kedua sumber antioksidan memberikan dampak yang positif terhadap komposisi susu. Suplementasi Vit E dan BTE meningkatkan terjadinya kelahiran kembar. Suplementasi Vit E menghasilkan *lambing rate* yang lebih tinggi dari BTE, tetapi tingkat kematian anak yang tertinggi diantara perlakuan. BTE menunjukkan bobot sapih domba yang lebih baik dibandingkan Vit E. Suplementasi BTE dan Vit E menghasilkan produktivitas induk yang sama pada periode laktasi. Kesimpulan dari penelitian ini adalah BTE lebih efektif digunakan sebagai sumber antioksidan untuk menjaga reaksi oksidasi pada PUFA daripada Vit E. Suplementasi Vit E pada pakan kaya PUFA menurunkan produksi susu, namun menghasilkan performa reproduksi yang sama dengan BTE.

Kata kunci: antioksidan, ekstrak teh hitam, induk domba, laktasi, reproduksi, vitamin E

*Corresponding author:
E-mail: dilla.mareistia@hotmail.co.id

INTRODUCTION

Nutritional levels of ewes in late pregnancy and lactation periods affected the energy reserves at born and lamb growth. Unfullfilled nutrient requirements for ewes affect placental size, foetal growth, foetal fat deposition, maternal udder development and milk production. Furthermore, it declined animal health and production performances. NRC (2007) recommends the ewes energy on late pregnancy is 1.5 times higher than maintenance. The fatty acids addition is one way to improve the feed energy. Hidayah *et al.* (2014) found that the supplementation of flaxseed oil protected with calcium soap resulted the high total volatile fatty acid which is indicating the highest supply of energy for ruminant. Our previous study indicated that non protected sunflower oil addition as the sources of essential poly unsaturated fatty acid (PUFA) improved reproductive performance of ewes, but the mortality rate was still high on birth and pre weaning periods. It is presumably due to the PUFA has not been used optimally.

The high metabolic demand in late pregnancy and lactation period also induced oxidative stress. Using high PUFA in the ration in these periods resulted in lipid peroxidation due to unbalanced condition between free radical and body antioxidant defense. Lipid peroxidation produces wide variety of aldehydes which can be formed as secondary products, such as malondialdehyde (MDA) (Ayala *et al.*, 2014). This compound is widely used to determine the oxidation process in various condition. Higher oxidative stress during pregnancy and lactation increase blood MDA concentration and it is implicated in some diseases which cause both of maternal morbidity and mortality (Bhale *et al*., 2014; Bhuyar & Shamshuddin, 2014; Yang *et al.*, 2011).

Exogenous antioxidant could be added to reduce the negative effect of lipid peroxidation formation on high PUFA ration. The role of antioxidants is to neutralize the excess of free radicals, to protect the cells against their toxic effect and contribute to health prevention (Rao *et al*., 2011). Antioxidant prevents PUFA from oxidative reaction (Murray *et al*., 1993), so it reach optimum PUFA utilization. Exogenous antioxidant, such as vit E is commonly used. Vit E supplementation on high PUFA ration improves lamb birth weight (Carper *et al*., 2005).

Local plants are rich in phytochemical compounds, which can contribute to maintain the quality of nutrition and health. The use of plant extracts to inhibit the oxidation process is a nutritional strategy that is very interesting to be studied. Shahidi (2009) stated that the natural antioxidant compounds of plant origin generally a polyphenol compounds which capable to maintain and inhibit reactive oxygen species (ROS) formation thereby reducing the risk on immunity. Wang *et al*. (2013) reported green tea polyphenols improved pregnancy rates in bovine embryos which associated with the increase of antioxidant enzyme genes expression and decreased apoptotic index in bovine blastocysts. Sgorlon *et al*. (2006) stated that tomato pomace (lycopene) and grape polyphenols were able to counter balance oxidative stress in ruminants, inducing specific transcriptional activity of genes involved in oxidant defense. Last investigation reported the multimeric polyphenols, theaflavins and thearubigins on black tea had more antioxidant abilities (Frei & Higdon, 2003), but rare study regarding its effect on ewes reproductive performances. The aim of this study was to compare the effect of vit E and BTE supplementation as antioxidant sources on high PUFA rations on the blood malondialdehyde (MDA) concentration, reproduction and lactation performance of Garut ewes.

MATERIALS AND METHODS

Animals and Diets

Twelve of 3-4 month gestations of Garut ewes were assigned into a completely randomized design with 3 treatments and 4 replications. The trial was lasted for 90 d. Ewes were housed in individual cages and had free access to drinking water.

Ewes were fed *Brachiaria humidicola* and concentrate containing 6% sunflower oil. Each concentrate on each treatment was added with different antioxidant sources, namely: Control= basal diet without antioxidant source, vit E= basal diet supplemented with 500 ppm vitamin E, BTE= basal diet supplemented 500 ppm (37.5 g/100g polyphenol) black tea extract. BTE was extracted from black tea leaves, using ethanol extract by maceration method (BPOM, 2004). Chemical composition of *B. humidicola* and concentrate is shown in Table 1.

Experimental Procedures

Ewes were offered rations twice a day with 1 kg/day *B. humidicola* (07.00 and 16.00 h) and *ad libitum* concentrate (refilled at 07.00, 12.00, and 16.00 h). The residual feed was collected from the feeder daily. Chemical composition of concentrate and forage were determined by AOAC (2005). Ewes were weighed at the beginning and further once a week along the experimental period. The average of daily gain (ADG) and daily feed intake (ADFI) were recorded. The protein and TDN intake were calculated.

Individual blood samples were taken in the morning before feeding, from the jugular vein into a vacutainer. The blood samples were centrifuged at 3000 rpm for 15 min. The serum was separated and placed into

Table 1. Chemical composition of concentrate and forage (dry matter basis)

Chemical composition	Concentrate	*Brachiaria humidicola*
Dry matter[a](%)	87.16	20.81
Ash[a] (%)	5.64	7.29
Crude protein[a](%)	20.41	12.88
Ether extract[a](%)	8.05	0.76
Crude fibre[a](%)	8.64	33.20
Nitrogen free extract[a](%)	57.26	45.86
Total digestible nutrient[b](%)	74.00	55.00

Note: [a]Analyzed values; [b]Calculated values according to Wardeh (1981).

Table 2. The fatty acid composition of concentrate and forage (g/100g of total fatty acids)

Fatty acids[a]	Concentrate	*Brachiaria humidicola*
Saturated fatty acids	40.98	9.19
Lauric acid (C12:0)	21.49	1.03
Myristic acid (C14:0)	8.26	0.46
Palmitic acid (C16:0)	7.23	4.50
Stearic acid (C18:0)	3.25	1.21
Arachidic acid (C20:0)	0.18	0.59
Behenic acid (C22:0)	0.40	0.68
Lignoceric acid (C24:0)	0.17	0.72
Unsaturated fatty acids	38.98	9.89
Mono-unsaturated fatty acids	16.02	0.94
Myristoleic acid (C14:1)	*	0.17
Palmitoleic acid (C16:1)	0.06	0.04
Oleic acid (C18:1)	15.96	0.73
Poly-unsaturated fatty acids	22.96	8.95
Linoleic acid (C18:2)	22.96	4.57
Linolenic acid (C18:3)	*	4.38

Note : *undefined; [a]Analyzed values

an Eppendorf tube then stored at -20 °C until analysis. Blood malondialdehyde (MDA) concentration was determined by Rice-evans & Anthony method (1991). Sampling and analysis were repeated on late pregnancy, lactation and weaning periods.

After birth, litter size, birth type, birth weight of lamb and lamb's mortality were recorded. The lambs were weighed within 24 h. Lambs were weighed every week from the day of birth. The ewes weight difference before and after lambing was defined as the pre-lambing live weight change (LWC).

Milk production was measured by the estimation method based on the weight gain of lamb until it was reached 28 days old. Six kg of milk consumption is needed to produce 1 kg of lamb daily gain (Dove, 1988). The calculation was as follows:

Milk production (g) = lamb average daily gain (g) x 6

Milk samples were collected from all ewes at 14, 28, and 56 d of parturition. At the end of sampling, milk was composited. Samples were analyzed for the contents of total protein, lactose and density by means of Milkotester Master Pro.

Statistical Analysis

The blood MDA concentration, feed intake, pre-lambing live weight change (LWC), post-lambing average daily gain (ADG) and milk production of ewes were analyzed by ANOVA using SPSS statistical software. Any means differences were analyzed by Duncan's multiple range tests. Milk composition and performance of ewes and lamb were analyzed using descriptive analyzes.

RESULTS AND DISCUSSION

Blood MDA Concentration

Late pregnancy and early lactation were the critical period where the body metabolism increased. Ewe's ration containing PUFA was also very susceptible to induce lipid peroxidation. Unbalanced condition between free radicals and body antioxidant defense develops oxidative stress which can decrease immunity and health (Falowo *et al*., 2014). Consequently, it needs more exogenous antioxidant.

Blood MDA concentration in ewes along the trial period was presented in Table 3. The MDA concentrations between treatment was similar, however the highest downward trend occurred when the ewes fed ration added BTE compared to the other treatments. Blood MDA was reduced 0.3% in the late pregnancy, 7.4% in early lactation and 18.2% in weaning period when vit E was added into the ration, but BTE gave better results. It reduced 2.9% blood MDA concentration in late pregnancy, 44% in early lactation and 30.3% in weaning period.

Vit E and polyphenol of BTE as antioxidant work in many different ways such as scavenging free radicals, chain-carrying species in the lipid peroxidation, breaking chain propagation and binding metal ions (Niki *et al*., 2005; Lee *et al*., 2015). In this study, BTE was more effective to protect feed PUFA from free radical damage than vit E. This result persumably due to BTE has higher antioxidant activity than vit E. Plant extract polyphenol exhibited better antioxidant properties than vit E (Brenes *et al*., 2005; Goñi & Serrano, 2005). So that the level of vit E on this study was not an effective level for supporting its antioxidant function.

Kuribayashi *et al*. (2010) stated that giving 600 mg/kg diet of vit E as antioxidant source could reduce blood MDA and depress blood vessels damage on rabbits due to oxidative stress caused by corticosteroid induction. The blood MDA was higher than those reported by Duygu *et al*. (2011) and Bijan *et al*. (2012) who examined the blood MDA of infected sheep on recovery period. Nevertheless, other factors such as environment, temperature, feed and method of determination affected the blood MDA concentration. In this study, BTE was more effective to protect feed PUFA from free radical damage than vit E.

Table 3. Blood MDA concentration on ewes fed different antioxidant sources along the trial period (mg/100mL)

Periods	Treatment		
	Control	Vit E	BTE
Pre-treatment	0.872±0.15	0.756±0.00	0.842±0.12
Late pregnancy	0.653±0.15	0.651±0.14	0.634±0.15
Lactation	0.983±0.12	0.910±0.26	0.545±0.01
Weaning	0.711±0.36	0.581±0.12	0.495±0.07

Note: Control= basal diet without antioxidant source, vit E= basal diet supplemented with 500 ppm vitamin E, BTE= basal diet supplemented 500 ppm (37.5 g/100g polyphenol) black tea extract; MDA= malondialdehyde.

Feed Intake, Pre-lambing Live Weight Change, and Post-lambing Average Daily Gain of Ewes

The DM intake was not significant among the treatments (Table 4). It indicated that supplementation of vit E and BTE on high PUFA rations had no adverse effect on ewe's feed intake. Our results agreed with NRC (2006) that recommended 600-900 g/head/d of DM intake for pregnant ewes with 20-30 kg body weight. Different antioxidant sources had no effect on CP and TDN. It could be because of the rations were formulated in *iso*-energy and *iso*-protein so ewes fed the similar amount of nutrients.

A similar finding was reported by Zain (2009) who found the native grass substitution with ammoniated cocoa pod on sheep resulted similar nutrient intake due to the chemical composition of rations almost the same. Tillman *et al*. (1989) stated that nutrient intake was affected by physical form and chemical composition of feed, provision frequency as well as anti-nutrients. Phenolic acids, flavanoids and tannins, the most important polyphenol in plants which have antioxidant properties also can act as anti-nutrient because of its ability to bind many nutrients and affect their bioavailabity (Jakobek, 2015). Its astringent taste produce a feeling of roughness, dryness in the palate which decrease the palatability. Though we didn't measure the tannin content of BTE, our present study showed that the tannin content on 500 ppm BTE did not affect feed intake. Some previous studies showed that the level of tannins, kind of polyphenol had no effect on feed intake (Xu *et al*., 2007; Salinas-Rios *et al*., 2015) while polyphenols were known to reduce feed intake by decreasing the palatability and/or ruminant turn over as well as the digestion, but it was influenced by the dosages.

There was no significantly different on pre-lambing LWC between treatments when antioxidant sources were given (P>0.05). This result was presumably due to the similar amount of DM intake. In current study, vit E supplementation tended to have the fewest pre-lambing LWC among others. This result indicates that the ewes on vit E treatment utilized feed nutrients for recovering their body condition in spite of producing milk. Antioxidant play an important role on protecting PUFA so it can be used as energy source and other function in the animal body. PUFA especially linoleic acid and α-linolenic acid are the precursors of long-PUFAs (eicosapentanoic acid and arachidonic acid) synthesised. These fatty acids are the precursors for eicosanoic including prostaglandin which related to the fast uterine involution after lambing and ewes fertility (Gulliver *et al*., 2012; Cerri *et al*., 2009; Coyne *et al*., 2008).

The high pre-lambing LWC on both BTE supplementation and control treatments presumably due to their high milk production (Table 5). The period from 3 wk before to 3 wk after lambing were the important stage that the energy requirements for milk production and maintenance exceed the energy from feed intake (Esposito *et al*., 2014). This condition induce the physiological state of energy balance then resulting the higher weight lose of ewes.

Ewe's post-lambing ADG was not affected by the different antioxidant sources supplementation. Nutrient requirement of ewes at lactation phase was higher than other phases because it was used for milk production. This caused the negative impact on ADG of ewes in early lactation. ADG will increase after peak of lactation due to the decrease of milk production, so the nutrients used for weight gain (Freer & Dove, 2002). The lowest ewes post lambing ADG on BTE treatment, presumably due to BTE treatment has higher milk production than vit E so the greater nutrient supply is needed. The nutrient was used to fulfill the requirement for producing milk, then its ADG declined. The nutrient supply didn't enought to provide the requirement to produce milk and increase ADG. In line with our study, Po *et al*. (2012) found that feeding Yerba mate (contained of polyphenol) dereased live weight in Dorper ewes in 1-7 wk of lactation period.

Table 4. Feed intake and live weight changes of ewes fed different antioxidant sources

Parameters	Treatment		
	Control (n=4)	Vit E (n=4)	BTE (n=4)
DM intake (g/head/d)			
Forage	194.75±73.36	170.62±49.30	163.65±37.89
Concentrate	593.90±31.15	598.32±28.02	635.32±29.21
Total	788.65±76.47	768.95±45.00	798.98±65.91
CP intake (g/head/d)	120.70±12.19	118.19± 7.21	125.72± 9.83
TDN intake (g/head/d)	596.78±62.09	580.19±65.43	573.98±49.06
Pre-lambing LWC (kg)	-7.50± 0.21	-6.20± 1.25	-8.50± 2.65
Post lambing ADG (g/head/d)	53.13± 8.55	51.79±19.09	44.05±12.40

Note: Control= basal diet without antioxidant source, vit E= basal diet supplemented with 500 ppm vitamin E, BTE= basal diet supplemented 500 ppm (37.5 g/100g polyphenol) black tea extract; LWC= live weight change; ADG= average daily gain; DM= dry matter; CP= crude protein; TDN= total digestible nutrient; n= the number of ewes.

Table 5. Ewes milk production and composition fed different antioxidant sources

Parameters	Treatment		
	Control (n=4)	Vit E (n=4)	BTE (n=4)
Milk production (g/head/d)	1125.00±81.13^{a}	629.00±107.85^{b}	1029.00±312.98^{a}
Milk composition			
Protein			
%	5.52± 0.58	6.15± 0.15	6.12± 0.14
g	62.47± 4.47^{a}	38.66± 6.63^{b}	49.07± 14.92^{b}
Lactose			
%	4.12± 0.15	4.77± 0.29	4.69± 0.03
g	46.63± 3.34^{ab}	38.47± 6.60^{b}	53.60± 12.28^{a}
Density (kg/m^3)	1.037	1.043	1.041

Note: Means in the same row with different superscript differ significantly (P<0.05). Control= basal diet without antioxidant source, vit E= basal diet supplemented with 500 ppm vitamin E, BTE= basal diet supplemented 500 ppm (37.5 g/100g polyphenol) black tea extract; n= the number of ewes. *Estimated values (Dove, 1988).

Milk Production

Different antioxidant sources affect amount of milk production ($P<0.05$). Table 5 shows that both BTE and control treatments had higher milk production than vit E. There was no difference in the protein and lactose concentration as well as the density of milk. The supplementation of vit E significantly produced lower quantity of milk protein and lactose than other treatments ($P<0.05$). However, vit E increased the protein and lactose concentration of milk about 11.41% and 15.78% respectively versus the control treatment.

In agreement with Mutoni *et al.* (2012) who stated that milk production was increased when 1000 IU of vit E was added on cows feed., but vit E supplementation on this study decreased the milk production (Table 5). The dosage of 50 mg vit E injection at day 30 and 15 prior to the day of parturition reduce the milk somatic cell counts and also the udder infections, which induce the milk production (Qureshi *et al.*, 2010).

Dosage of vit E as antioxidant source plays an important roles to maintain the oxidation balance in animal body. Level of vit E supplementation in our current study has not been able to prevent the amount of ROS which associated with mastitis disease and the decline of milk production (Celi, 2010). The reaction between ROS and cellular macro molecules, such as PUFA enhanced ephitelial abrassion and apoptosis on the udder (Suriyasataphorn *et al*., 2006). Furthermore, PUFA oxidation reduced the energy supply resulting the low milk production. Our results were in accordance with Pirestani *et al.* (2014) who found the supplementation of vit E and Se resulted less milk production than control treatment in cows.

The lower milk production also affected milk protein and lactose quantity. However its concentration as well as milk density were increased. This results might also have been the consequences of a lower milk production. Barrón-Bravo *et al.* (2013) found that goats with higher milk somatic cell score (indicating mastitis diseases) produced low milk yield and fat content, but higher protein content.

The BTE supplementation had similar milk production than control, but it also tended to have higher protein and lactose content as well as milk density than control. This result confirmed that BTE as a source of antioxidant maintained the oxidation balance in animal body. BTE contains of polyphenol which has antioxidant activity, such as catechin and theaflavin (Min *et al*., 2013; Carloni *et al.*, 2013). Those compounds protected PUFA thus providing energy source and precursors of steroid hormones (glucocorticoids, progesterone and estradiol) which will stimulate the secretory cells of the mammary gland to increase milk production. Higher milk production is related to lactose levels due to lactose synthesis is the principal factor that determines the milk volume (Po *et al.*, 2012). In our study, high milk production also influenced higher pre-lambing LWC and decreased ewes post-lambing ADG.

The BTE supplementation had higher milk protein and lactose than vit E. BTE polyphenol act as antioxidant prevent the oxidative status and retain the health of the mammary gland. It also increased the permeability of alveolus cell of mammary gland (Nurdin, 2004; Nurdin, 2007) thus increased the transfer of serum proteins into the mammary gland. Polyphenol also maintained the balance of rumen microbes (Nurdin, 2004). Protozoa, as bacteria predators, become the most deleterious effect on the efficiency of N use in the rumen. Antioxidant properties from polyphenol will change the microbes balance in the rumen by rid the pathogenic microbes so that the number of bacteria increase (Mardalena *et al.*, 2014). This case will enhance microbial protein synthesis then increase amino acid flow to duodenum (Mueller-Harvey, 2006). Moreover, it supplied amino acids contributing milk protein synthesis.

Polyphenol also prevent PUFA as energy source so it can supply glucose to mammary gland which is important to produce milk lactose (Ramli *et al.*, 2009). Aguiar *et al.* (2014) found that feeding phenolic compounds from propolis extracts had no effects on milk production of Holstein cows but increased the lactose and protein yield than control. Our results may also be attributed to the high concentrate diets which lead to decrease milk fat content because of the changes in fat synthesis (Chouinard *et al.*, 1999; Loor *et al.*, 2010). But our current study could't support this opinion, due to no milk fat data.

Ewes and Lamb Performances

The performance of ewes and lambs was reported in Table 6. Overall, the supplementation of antioxidants improved the lambing rate, the number of twin birth,

Table 6. Ewes and lamb performances fed different antioxidant sources

Parameters	Treatment		
	Control	Vit E	BTE
The number of ewes (head)	4	4	4
Lambing rate (%)	150	225	175
Type of birth (%)			
Single	50	25	50
Twin	50	75	50
Mortality rate of twin lamb (%)	0	44.44	14.29
Birth weight of lamb (kg)			
Single	2.70 (n=2)	2.40 (n=1)	3.20 (n=2)
Twin	2.10 (n=4)	1.49 (n=8)	1.60 (n=5)
Total	13.8	14.32	14.4
Weaning weight of lamb (kg)			
Single	10.60 (n=2)	7.40 (n=1)	10.60 (n=2)
Twin	6.85 (n=4)	5.55 (n=4)	6.45 (n=4)
Total	48.6	29.6	47
Weight gain of lamb (g/head/d)			
Single	141.07 (n=2)	89.29 (n=1)	132.14 (n=2)
Twin	84.82 (n=4)	61.61 (n=4)	84.82 (n=4)
Total	621.42	335.73	603.56

Note: Control= basal diet without antioxidant source, vit E= basal diet supplemented with 500 ppm vitamin E, BTE= basal diet supplemented 500 ppm (37.5 g/100g polyphenol) black tea extract.

and the birth weight of lambs. This result could indicate their function in reducing the oxidative stress on the animals and protect feed PUFA. PUFA is essential to produce progesterone in the body. It plays a role in protecting and maintaining the pregnancy, so that the embryo can well develop into a fetus and born safely. Gulliver *et al*. (2012) stated that PUFA reduced the PGF2α synthesis and continued with progesterone secretion that might improve embryo survival and reduced embryo mortality.

Table 6 shows that vit E increased lambing rate and the case of twin birth. PUFA from sunflower oil influenced reproduction by supplying precursors of steroid hormones. Prostaglandin, one of steroid hormones is an important mediators of ovulation. Supplementation of antioxidant sources prevents PUFA from ROS oxidation so it can be used as energy source for supporting a multiple birth. Antioxidant supplementation can increases the antioxidant status of the reproductive tract which improve the fetal development on late pregnancy phase. Diet enriched with long chain n-3 PUFA resulted high ova release (Trujilo *et al*., 1995). El-Nour *et al*. (2012) stated that PUFA from calcium soap of fatty acid supplementation increased ovulation rate, follicle number and the pregnancy rate. Vit E could prevent negative effect of noise on the pregnancy rate and the rate of fetal death and abortion on female mice (Fathollahi *et al*., 2013).

The supplementation of BTE on ewes ration improved the lamb birth weight on single birth (Table 6). Antioxidant improved ewes immunity along the pregnancy period and supported the fetus development as well as increased the lamb birth weight. Twin birth was increased when vit E and BTE were added into the ration. Ewes with twin birth tended to produce lower lamb birth weight. This result is in agreement with Yilmaz *et al*. (2007). The low birth weight of twin lamb is most likely because there was competition between twins for placental nutrients and mothers milk when they were in maternal environment. Gardner *et al*. (2007) stated that birth weight was not only affected by the nutrient supply from mother to multiple litters, but also affected by the capacity of mother to bear multiple litters, forces in uterus and the fetal genotype. Maternal uterine space has optimum capacity to gestate offspring. If the litter size increase, the birth weight will be decline.

Antioxidant supplementation resulted in the upward trend on lamb mortality (Table 6). The higher mortality rate could be caused by the higher number of twin lambs than control which is resulting the longer birth process and the lower lamb weight. The case of mortality was higher in vit E than BTE. One ewe fed ration with vit E supplementation delivered 4 lambs but they were died due to the lower birth weight (0.8-0.9 kg/head). In the similar case, a lamb (1.2 kg/head) was died when delivered process of ewes fed ration with BTE supplementation. Both antioxidant supplementations were given on the late pregnancy period so that we can not control the number of embrio. We infered that vit E can not protect PUFA from oxidation process, then the energy supply did not fulfilled with the energy requirement to support the development of more than two fetuses.

Chniter *et al*. (2011) reported that mortality rate in single birth lambs was low. Higher mortality may be associated with the low birth weight of lambs from large litter size. After birth, survival of the newborn depends largely upon the interactions quality with the mother, the amount of available milk and the competition with siblings as well as diseases (Nowak & Poindron, 2006). The competition in obtaining colostrums on twin lambs made them not get enough nutrients on early birth and then decreased the survival rate .

Growth of lamb is largely dependent on the nutrients of milk from their mother during 4 weeks of life. The higher weaning weight of lamb suggest the better lactation performances of ewes. Table 6 showed that BTE treatment had similar body weight and weight gain of single and twin lambs at weaning period, and vit E had the lowest one. It is likely that lambs growth was affected by dietary treatments. Fereira *et al.* (2014) found the increased of weaning weight was linear with the milk production of ewes. There was a relationship between the amount of ewe's milk production and weaning performances of lamb in the pre-weaning phase (Aroujo *et al.* 2008; Jarmuji, 2010).

This study found that vit E supplementation produced less milk than others. The low milk production could be the reason for the low body weight and weight gain of lamb on vit E supplementation treatment. Amount of milk production associated with the lamb's opprotunity to consume immunoglobulin and antioxidant. A healthy immune system of mother's will be transferred to the child through placenta and breast milk, including colostrum. Milk that contained enhancement growth factor and immunoglobulin will affect the health and growth rate of the offspring (Akbar, 2013).

Despite of higher milk production in BTE supplementation, the higher weaning weight of lamb could be caused by the polyphenol content in milk. Po *et al.* (2012) stated that milk production and its quality are critical factors for the survival and growth of lamb. Di Trana *et al*. (2015) found the positive correlation between polyphenol intake and milk polyphenol content in goats. Increasing level of antioxidant in parent's ration resulted high antioxidant content in milk. Antioxidant act as a radical scavenger, hydrogen and electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor as well as metal-chelating agents (Lobo *et al.*, 2010). It plays an important role in immune responses, thus increase the growth rate of lambs.

CONCLUSION

The supplementation of black tea extract was more effective to decrease blood malondialdehyde concentration of ewes. However, black tea extract and vitamin E supplementation gave similar respond to ewes reproductive performances. Vitamin E supplementation reduced the milk production of ewes.

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