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Research Article
Productive and Reproductive Performances and Blood Profile of Bali Cows Supplemented with Calcium Soap-soybean Oil

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Abstract

Objective: This study was aimed to evaluate the effect of calcium soap-soybean oil on productive and reproductive performances as well as blood profile of Bali cattle. **Materials and Methods:** This study used 12 heads of Bali cattle with the average body weight of 230 kg at Sobangan Breeding Centre, Bali Province, Indonesia. There were 2 dietary treatments i.e., control (no supplementation of calcium soap-soybean oil) and control+calcium soap-soybean oil 5%. Control diet which consist of Napier grass and concentrate with ratio approximately 80:20 and all treatment diets were offered for 60 days. All cows were oestrous synchronised using PGF2α injections and continued with artificial insemination. Variable measured were feed intake, body weight gain, average daily gain, feed efficiency, service per conception, blood nutrient and haematology. **Results:** The results showed that the addition of calcium soap-soybean oil significantly increased ($p<0.05$) feed consumption of forage and concentrate, but slightly decreased body weight gain and feed efficiency compared to the control treatment. Moreover, the addition of calcium soap-soybean oil significantly ($p<0.05$) enhanced reproductive performance of Bali cows such as pregnancy rate and proportion of cattle with service per conception (S/C) = 1. However, the use of calcium soap-soybean oil did not affect blood metabolites and blood haematology except white blood cells and leucocyte differentiation such as neutrophil, lymphocytes and monocyte. **Conclusion:** The use of calcium soap-soybean oil has positive effect on reproductive performance without altering the health status of Bali cows.

Key words: Bali cattle, calcium soap-soybean oil, reproduction, blood profile, blood metabolite

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bali cattle is one of the Indonesian indigenous beef cattle which has potency to be developed in beef cattle industry. Bali cattle has some excellence characteristics including high percentage of carcass, meat quality, high adaptability to tropical environments and can utilize low-quality feed resources. However, some problems associated with Bali cattle farming are the slow growing calves with low daily weight gain of about 0.3 kg/day and low reproductive performance[1]. One strategy to improve the reproductive performance of Bali cattle is the use of high quality ration usually energy prior to the start of the critical phase of reproduction such as start of breeding, before and after calving and suckling calves. A good nutrition during these critical stages also have direct impact on the ability of the cattle to rebreed in a right time.

In order to fulfill high energy requirement prior to the calving, the use of fatty acid sources in the ration may be the appropriate strategy. Supplementation of unsaturated fatty acids from vegetable oils in ruminant feed could improve the productive and reproductive performance of ruminants. However, the use of vegetable oils as unsaturated fatty acids sources need to be protected to avoid biohydrogenation process by rumen bacteria which convert unsaturated fatty acid to saturated fatty acid. Feed ingredients derived from vegetable oils contain high unsaturated fatty acids, such as oleic and linolenic acids that will undergo biohydrogenation process or saturation reaction massively in the rumen which could increase the saturated fatty acids in the rumen[2]. Moreover, vegetable oil is also a source of alternative energy needed by cattle. Furthermore, the use of vegetable oil as ruminant feed must be restricted because of some disadvantages effect in the rumen system. According to Bunting *et al.*[3], oil feeding above 5% in the ruminant diets may disrupt the microbial population in the rumen and reduce the ability of ruminants in the feed digestion.

To overcome this problem, addition of vegetable oil need to be protected to avoid biohydrogenation process by rumen bacteria. Calcium soap is a protection method of unsaturated fatty acids that can be easily and cheaply applied. Calcium soap is a chemical process to produce soap from fat and alkali materials known as saponification and added with calcium chloride[4].

Reproductive inefficiency has a significant impact on the economic performance of beef cattle production. Nutrition has an important role in reproduction. In addition, inadequate dietary energy intake and poor body condition can negatively affect reproductive function. Previous study showed that polyunsaturated fatty acids (PUFA) supplementation might enhance beef cattle fertility independent of their role as energy substrates[5]. Lipid supplementation has been used to increase the energy density of the diet and may also have direct positive effects on reproduction in beef cattle. Several different fatty acid sources have been studied to improve reproductive function[6]. Vegetable oils appear to have the greatest impact on reproductive performance such as soybeans oil[7].

The aim of this study was to evaluate the effect of calcium soap-soybean oil on productive and reproductive performances as well as blood profile of Bali cows.

MATERIALS AND METHODS

Animal and feeding treatment: This study used 12 heads of Bali cows with the average body weight of 230 kg. This study was conducted at Sobangan Breeding Centre, Bali Province, Indonesia. There were 2 treatments i.e., control (no supplementation of calcium soap-soybean oil) and control+calcium soap-soybean oil 5%. Control diet consisted of Napier grass and concentrate with the ratio approximately 80:20 and all treatment diets were offered for 60 days. Nutrient composition of Napier grass and concentrate ration are shown in Table 1. All cows were oestrous synchronized using PGF2α injections (6 cc of each cow) and continued with artificial insemination.

Variable measured were feed intake, body weight gain, average daily gain, feed efficiency, service/conception, blood metabolites and haematology.

Blood sampling: Blood samples were collected by jugular venipuncture. Blood was collected via coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL), placed on ice immediately, maintained at 4°C for 24 h and centrifuged at 3,000 rpm for 10 min at room temperature for serum collection. Variable measured were blood haematology, cholesterol, glucose, triglycerides, total protein, albumin and urea.

Statistical analysis: All data were analyzed by t-test and performed by using SPSS 13.0 for windows.

Table 1: Nutrient composition of Napier grass and concentrate ration

Nutrient (%)	Napier grass	Concentrate ration
Dry matter	23.60	88.47
Ash	10.67	9.82
Crude protein	13.13	3.38
Ether extract	3.64	10.23
Crude fiber	6.96	26.25
Nitrogen free extract	45.51	50.32

RESULTS

Feed intake, daily gain and feed efficiency: The addition of calcium soap-soybean oil significantly ($p<0.05$) increased the feed consumption of forage and concentrate, but significantly decreased body weight gain and feed efficiency compared to the control treatment (Table 2).

Reproductive performances of Bali cows: The addition of calcium soap-soybean oil significantly enhanced reproduction performance of Bali cows such as pregnancy rate and percent of cattle with S/C = 1 (Table 3).

Table 2: Feed intake, body weight gain and feed efficiency of Bali cows supplemented with calcium soap-soybean oil during 30 days

	Treatments	
Variables	Control (C)	Control+Ca soap soybean oil
Average dry matter intake (kg h^{-1}/day)		
Napier grass	4.31±0.20	4.32±0.08
Concentrate	0.88±0.00[b]	1.11±0.02[a]
Total	5.19±0.201[b]	5.43±0.09[a]
Body weight gain for 30 days (kg h^{-1})	9.60±5.68[a]	6.40±3.36[b]
Average daily gain/ADG (kg h^{-1}/day)	0.32±0.19	0.21±0.11
Feed efficiency	0.06±0.04	0.04±0.02

Feed efficiency: Average daily gain/average dry matter intake

Table 3: Reproductive performance of Bali cows supplemented with calcium soap-soybean oil for 30 days

	Treatments	
Variables	Control (C)	Control+Ca soap soybean oil
Average number of synchronization	1	1
Pregnancy rate (%)	50	83
Percent of cattle with S/C* = 1 (%)	50	83

*S/C: Service per conception

Table 4: Blood haematology of Bali cows supplemented with calcium soap-soybean oil for 30 days

	Treatments	
Variables	Control (C)	Control+Ca soap soybean oil
RBC (10^6 μL^{-1})	5.1817±0.4106	5.3717±0.7496
HGB (g dL^{-1})	10.617±1.2057	11.217±1.5250
HCT (%)	30.717±4.3847	30.883±4.4951
MCV (fL)	59.450±8.7697	57.850±7.3978
MCH (pg)	20.533±2.1144	21.000±2.3740
MCHC (g dL^{-1})	34.717±2.0971	36.383±1.5118
White blood cell (10^3 μL^{-1})	7.1317±0.7992[b]	10.192±1.9588[a]
Leucocyte differentiation (10^3 μL^{-1})		
Neutrophil (10^3 μL^{-1})	0.2650±0.1468[b]	0.5167±0.1800[a]
Lymphocytes (10^3 μL^{-1})	6.1050±0.8038[b]	8.6917±1.8851[a]
Monocyte (10^3 μL^{-1})	0.5167±0.1401[b]	0.7333±0.1442[a]
Eosinophil (10^3 μL^{-1})	0.1117±0.1107	0.0717±0.0857
Basophil (10^3 μL^{-1})	1.1283±2.4659	0.6367±0.1450

Blood haematology: The addition of calcium soap-soybean oil did not affect the concentration of red blood cell, haemoglobin, HCT, MCV, MCH and MCHC. In contrast, the addition of calcium soap-soybean oil significantly increased white blood cell and its differentiation i.e., neutrophil, lymphocyte and monocyte (Table 4).

Blood metabolites: The use of calcium soap-soybean oil did not affect the concentration of blood glucose, protein total, albumin, urea, cholesterol total, HDL, LDL and triglyceride (Table 5).

DISCUSSION

The increase of feed intake in calcium soap-soybean oil supplemented group indicates that calcium soap-soybean oil addition did not alter palatability of the ration. Although, calcium soap-soybean oil production was done using saponification method, they did not reduce feed consumption.

The addition of calcium soap-soybean oil increased feed intake, however, body weight gain and feed efficiency were reduced. It is known that the increase of body weight gain inline with the increasing of feed intake. Pavan *et al.*[8] suggested that the addition of corn oil could improve body weight of cattle up to 0.31 kg^{-1} corn oil consumption. Although, the use of corn oil decreased dry and organic matter digestibility, but daily body weight gain and total body weight improved with the increasing of corn oil level addition. In the current study, body weight gain of Bali cattle decreased with the addition of soybean oil calcium soap.

Reduction of body weight gain of Bali cows with the supplementation of soybean oil-calcium soap might be caused by the utilization of energy which was generated from soybean oil for improving reproductive performance instead for body weight gain[9]. Fat supplementation can be used to increase energy sources and will have positive impact on

Table 5: Blood metabolites of Bali cows supplemented with calcium soap-soybean oil during 30 days

	Treatments	
Variables	Control (C)	Control+Ca soap soybean oil
Glucose (mg dL^{-1})	46.000±7.9750	46.667±4.5019
Total protein (g dL^{-1})	8.1333±0.6743	8.0167±0.6369
Albumin (g dL^{-1})	3.0500±0.3450	3.2000±0.2608
Urea (mg dL^{-1})	18.967±3.7044	16.100±4.7628
Total cholesterol (mg dL^{-1})	156.83±18.258	148.67±49.184
HDL l (mg dL^{-1})	109.98±13.030	103.07±37.230
LDL l (mg dL^{-1})	24.783±10.591	23.367±14.661
Triglyceride (mg dL^{-1})	110.33±12.941	111.17±11.053

reproductive performances of beef cattle. Bilby *et al.*[10] reported that continuous supplementation of fat in the feed could be used to manipulate fatty acids content in the tissue of beef cattle including embryonal and reproductive tissues.

Feed efficiency reduced inline with the decreasing of body weight gain. The reduction of feed efficiency means that the energy generated from calcium soap-soybean oil in reproductive phase might be mostly used for reproduction. Low feed efficiency may also be influenced by the effect of soybean oil on rumen microbial activity. Feed efficiency depends on the ability of rumen microbes in degrading feed in the rumen. Bessa *et al.*[11] reported that body weight gain of sheep was reduced with feed intake of concentrate containing too high soybean oil (121 g kg^{-1} feed) for long period of time.

There are several studies reporting a better reproductive performance in cows fed lipid supplementation. In this respect, Staples *et al.*[12] showed that lipid consumption exerted a positive effect on reproductive aspects in dairy cows. In beef cattle, the same trend has been observed. De Fries *et al.*[13] reported that Brahman cows consuming 5.2% lipids in the ration showed a trend towards an increase in pregnancy rate than those cows which consumed only 3.7% lipids in the ration. Ferguson and Leese[14] observed a 2.2 times increase in the possibility of pregnancy in lactating cows consuming 0.5 kg lipids/day. In another study, it was demonstrated that grazing cows supplemented with fat, pregnancy rate at first service was 16% higher than in cows which did not receive fat in the ration[15].

Supplementation of calcium soap-soybean oil into concentrate for 30 days did not affect the oestrus synchronization in Bali cattle. The percentage of calving was linear to the percentage of pregnancy. It means that calcium soap-soybean oil supplementation affected reproductive performances of Bali cows. Fat supplementation can increase conception rate, pregnancy rate and reduce service period[16]. Lopes *et al.*[5] reported that supplementation of Ca salt soybean oil after artificial insemination, during the luteolysis period and early pregnancy can increase pregnancy rate in *Bos indicus*. In addition, Graham *et al.*[17] said that the use of whole soybeans in the ration of adult beef cattle for 30 or 45 days before calving can increase the first service conception (62.8 vs. 85.7% and 62.5 vs. 75%). Moreover, Abu El-Hamd *et al.*[18] reported that the supplementation of protected oil into the feed shorten the service interval, service period and estrus detection, lower service per conception and better conception rate compared to control.

Calcium soap protection of soybean oil increased the post ruminal content of unsaturated fatty acids leading to the increase of its absorption. The increased of its absorption would affect the reproductive hormones, so that increasing the pregnancy rate of Bali cows. Soybean oil contains high unsaturated fatty acids especially linoleic acid (51%). Hess *et al.*[19] reported that supplementation of feed containing high linoleic acid at postpartum period will increase cows reproductive performance. The addition of fat into the ration of beef cows during the late pregnancy period can increase the pregnancy percentage (91.6%) compared to control (82.9%). High poly-unsaturated fatty acids (PUFA) in the reproductive tissues will affect the corpus luteum (CL) and endometrium[20], whereas, according to Grant *et al.*[21], feed containing PUFA influences reproductive hormones such as prostaglandin and progesterone. Cooke *et al.*[22] reported that linoleic acid and omega 6 in PUFA increases in the reproductive tissues of beef cows with the supplementation of Ca salt soybean oil at the amount of 100 g. Omega 6, omega 3 and part of arachidonic acid are precursor of PGF2α synthesis. The PGF2α is a hormone that influences luteolysis and pregnancy. Supplementation of omega 3 can inhibit the synthesis of PGF2α, luteolysis process and reduce pregnancy loss[23,24].

The supplementation of calcium soap-soybean oil did not increase red blood cells, but it increased white blood cells and leucocyte differentiation of Bali cows. Calcium soap protection of unsaturated fatty acids increased blood linoleic acid. Linoleic acid affected the content of arachidonic acid that could increase eicosanoid content which has a role in mediating the prostaglandin inflamation, leukotriene and several metabolites. The increased of linoleic acid will increase the leucocyte content of the blood. James *et al.*[25] reported that linoleic consumption from corn oil, soybean oil and sunflower oil can produce leucocyte 10-16% compared to linolenic acid (0.1-0.3%) and oleic acid (0.1%). Neutrophil, lymphocyte and monocyte increased compared to the other leucocyte cells. However, red blood cells and white blood cells number were still in the normal range. According to Pal and Bhatta[26], number of red blood cells in cross bred Holstein Friesian (HF) cows are $6.32\text{-}11\times10^{6}$ mm^{-3}, white blood cells ($8.2.13.8\times10^{3}$ mm^{-3}), neutrophil (25-40%), lymphocyte (39-67%) and monocyte (2-3%). According to Calder[27], monocyte and macrophage produce PGE_2 and PGF_2 in large amount, neutrophil produces PGE_2 and mastocyte cells in a moderate amount to produce PGF_2.

Otto *et al.*[28] reported that glucose and cholesterol concentration in the blood of cattle fed crude degummed canola oil (CDCO) are not significantly different from control (without the addition of CDCO). The addition of oil compared to control does not influence concentration of serum glucose, cholesterol and protein, but increases the serum triglyceride content.

The supplementation of calcium soap-soybean oil also decreased blood LDL and HDL content although it was not statistically significant. The reduction might be caused by high content of unsaturated fatty acids in the body. Decreasing level of LDL and HDL in the blood showed that the supplementation of soybean oil in the form of calcium soap protected was not detrimental to Bali cows health. According to Stewart *et al.*[29], unsaturated fatty acids in the feed can be precursors of saturated fatty acids, so that HDL concentration in the plasma reduced. The addition of calcium soap-soybean oil also reduced blood plasma LDL, consequently decreasing the level of blood cholesterol because LDL level is inline with blood cholesterol level. Low LDL in the blood reduces cholesterol transportation into the blood. Supplementation of calcium soap-soybean oil also slightly increased blood triglyceride. Triglyceride will be used as energy source. According to Shahriar *et al.*[30], unsaturated fatty acids in the soybean oil can increase digestibility and absorption as well as triglyceride biosynthesis in the liver, so that free fatty acids concentration will increase in the blood.

CONCLUSION

The addition of calcium soap-soybean oil significantly increased dry matter intake but decreased body weight gain and feed efficiency of Bali cows. In contrast, the addition of calcium soap soybean oil significantly enhanced reproduction performance of Bali cows such as pregnancy rate and percent of cattle with S/C =1. However, the use of calcium soap-soybean oil did not affect blood metabolites and blood profile except white blood cells and its differentiation. In conclusion, the use of calcium soap-soybean oil has positive effect on reproductive performance and does not alter the health status of Bali cows.

SIGNIFICANCE STATEMENT

This study discovers the feed supplement which contain protected unsaturated fatty acid from soybean oil that can be beneficial for reproductive performance of Bali cattle. This study will help the researcher to uncover the critical areas of nutrient/energy deficient in reproductive phase that many researchers were not able to explore. Thus a new theory on protected unsaturated fatty acid supplementation may be arrived at.

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