Buletin Peternakan 44 (2): 20-26, May 2020

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v44i2.55338

The Effect of Choline Chloride Supplementation on the Reproductive Performance of Simmental Bulls Fed Protected Protein in the Ration

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Article history
Submitted: 13 April 2020
Accepted: 26 May 2020

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ABSTRACT

SOYXYL supplementation as a source of high-quality protected protein in rumen (Rumen Protected Protein) has proven to improve reproduction performance of bulls. The SOYXYL is an extrusion product of soybean (*Glycine max*) as a source of rumen protected protein. The potential of the protein supplementation is maximally expressed when it is supported by sufficient of metabolic rate. In this case the choline chloride supplementation increases the metabolic rate. The aim of the research was to analyze the effect of choline chloride supplementation on reproduction performance and hematologic status related to reproduction performance of bulls fed protected protein in the ration. The materials used were 12 bulls aged 5 years having weight of 700 ± 2.75 kg that were divided into 4 treatment groups, each of which consisted of 3 heads. The treatments applied were T0 (control): ration (grass + commercial concentrate) + protected protein supplement "SOYXYL"; T1: control + choline chloride 0.145% dry matter (DM) concentrate/head/day; T2: control + choline chloride 0.290% DM concentrate/head/day; T3: control + choline chloride 0.435% DM concentrate/head/day. Variables measured were nutrient consumption, blood lipid status, blood protein status, and reproduction performance. Data were analyzed using ANOVA in a completely randomized design and statistically processed using SAS program. The result showed that choline chloride supplementation by 0.435% from DM concentrate increased ($p<0.05$) the concentration of blood plasma protein, blood testosterone hormone, sperm concentration, and sperm motility from 6.44 g/dL, 4.66 ng/mL, 1006.74 million/mL, and 36.00%, respectively, in T0 to be 8.57 g/dL, 9.07 ng/ml, 1270.41 million/mL, and 70.00% in T3, respectively. The choline chloride supplementation up to 0.435% from DM concentrate to bulls supplemented with protected protein containing in the ration increased reproduction performance.

Keywords: Bulls, Choline chloride, Protected protein, Sperm concentration, Testosterone

Introduction

Reproductive performance of bulls in producing high quantity and quality of semen is closely related to the reproductive performance of cattle in terms of conception rate and calving interval. Among the factors that affect the reproductive performance of bulls are the quality and quantity of feed provided. The quality of feed, especially concentrate, has received enough attention, but its increased utility has not received adequate attention. In practice, most of concentrate composition used in bull ration is technically acceptable. However, the efficient use of nutrients in the concentrates (especially proteins) has not been fully considered. Meanwhile, efforts to improve the efficient use of the nutrients have been developed, including the use of rumen-protected proteins.

Although it has been proven that rumen-protected protein supplements, such as SOYXYL, are beneficial for beef cattle fattening (Prasetiyono *et al*., 2007), further investigations need to be carried out especially the ones focusing on bull's reproductive performance. Field observations about the effect of adding SOYXYL as one of the rumen-protected protein supplements identified that improvements in the reproductive performance of bulls could also be improved by adding feed additives; for example, choline chloride which functions to stimulate biosynthetic processes at the level of metabolic intermediaries which leads to an increase in the quality and quantity of spermatozoa.

Choline chloride influences intermediary metabolism through its role as methyl donor to synthesize limiting essential amino acid (methionine), phosphatidylcholine, and carnitine (Chandler and White, 2017). As choline chloride is rapidly degraded in the rumen, its supplementation in diet is not effective; therefore, it should be supplemented in the form of protected choline chloride (Chaudhari *et al*., 2017). Methionine is an amino acid that is needed in every protein biosynthesis (Gorissen *et al*., 2018); meanwhile, phosphatidylcholine is needed as a component of bio-membrane that stimulates enzyme to activate intracellular metabolism, and it also functions as an essential lipoprotein component in transporting lipid (Lagace, 2016). In addition, carnitine has an important role in transporting lipid acid into mitochondria in order to provide energy in the biosynthesis processes. The mechanism by which choline chloride can increase the effectiveness of the use of protected protein nutrients in intermediate metabolism, that is, choline chloride can increase the metabolic rate leading to an efficient use of protein (Grummer, 2016). It is therefore expected that the increased efficiency of protein used due to SOYXYL supplementation will be much higher by combining the supplement with choline chloride supplementation. Based on that mechanism choline chloride is expected to improve the reproductive performance of bulls.

Given the explanations, the objective of this study was to investigate the effect of choline chloride supplementation on the reproductive performance of Simmental bulls fed protected protein in the ration. The choline chloride improves the reproductive performance of bulls by increasing the metabolic rate.

Materials and Methods

Experimental bulls

Twelve Simmental bulls aged approximately five years with body weight of 700±2.75 kg were reared in the Artificial Insemination Institute of Central Java Province for 90 days. All bulls were individually stalled. Prior to experiment, all healthy bulls were injected with preparative vitamin containing of A, D, E, and K vitamins and adapted to the treatment feed for a month.

Experimental procedure

All experimental bulls were fed with elephant grass (15% DM) and drinking water *ad libitum*, commercial concentrate (containing 85.25% of DM, 3.6% ash, 2.22% crude fat, 16.5% crude fiber, 71.26% Total Digestible Nutrients, 18.23% crude protein, and 33.35% Un-degraded Dietary Protein, on DM basis) as many as 5 kg/head/day, protected protein supplement "SOYXYL" (Prasetiyono *et al.*, 2007) as many as 3% from the concentrate portion (Prasetiyono *et al.*, 2020), and choline chloride 60% (manufactured by Shandong NB Technology Co., LTD., China). The levels of choline chloride used were 0.145%, 0.290%, and 0.435% from DM concentrate/head/day.

Feeding was scheduled twice a day, morning (7 AM) and afternoon (3 PM), and the experimental bulls were divided into four treatment groups; T0, T1, T2, and T3. T0 (control) = ration (grass + commercial concentrate) + protected protein supplement "SOYXYL". T1 = control + choline chloride 0.145% DM concentrate/head/day. T2 = control + choline chloride 0.290% DM concentrate/head/day. T3 = control + choline chloride 0.435% DM concentrate/head/day. All treatments were replicated three times and ran according to completely randomized design (Steel *et al*., 1996).

Variables observed were nutrient consumption, blood lipid status, blood protein status, and reproduction performance. Semen and blood used for the experiment were collected at the end of the experiment (90^{th} day) from 7 - 8 AM. The semen was collected using an artificial vagina.

Sperm motility was calculated by observing spermatozoa cells movement using microscope and stated in percentage (%). The percentage of the spermatozoa motility was calculated by (∑ total spermatozoa – unmovable spermatozoa) X 100%. Sperm concentration was calculated using hemocytometer (million/mL).

Diluted and homogenized fresh sperm was sucked using erythrocyte pipet and were dropped into hemocytometer; then, they were observed using microscope as they appeared within the hemocytometer. Five diagonal boxes were chosen within which the number of spermatozoa were calculated. Sperm concentration was calculated by the number of spermatozoa in the boxes X 10^6/million/mL (Vijayalakshmy *et al*., 2018).

The level of blood protein was analyzed three hours after feeding experimental bulls. Sterile disposable sterile syringe, Eppendorf tube, and spectrophotometer were used to collect and analyze blood samples. The blood samples were collected at 90^{th} day of the experiment. The blood collected, 3 cc – 4 cc, was taken from a jugular vein using Venoject tube containing EDTA that functions to avoid blood being coagulated. After that, the collected blood was kept in a cool box, brought to laboratory, and centrifuged with 3000 rpm for 10 minutes in order to take the blood plasm to be analyzed its level of protein.

The level of total protein concentration of the blood sample was calculated by using photometer principles (Photometer 5010®) using commercial kits. The fundamental principle of photometry is measuring light absorption as a result of light interaction that has a certain wavelength with substances or color substances the light through in. Meanwhile, testosterone hormone was analyzed using Eliza method (the enzyme linked immunosorbent assay) using testosterone kits (Sachidhanandam *et al*., 2010).

Statistical analysis

Data were analyzed statistically by analysis of variance (ANOVA) using the general linear model procedures of SAS (SAS, 2009), and Duncan's Multiple Range Tests were then used when the significant differences ($p<0.05$) appeared between treatment groups (Steel *et al*., 1996).

Results and Discussion

Nutrient consumption

Data on the nutrient consumption of bulls are presented in Table 1. The elevated levels of choline chloride supplement significantly increased ($p<0.05$) consumption in terms of the levels of Dry Matter (DM), Crude Protein (CP), Nitrogen Free Extract (NFE), Total Digestible Nutrients (TDN), and Ether extract (EE). It was apparent that DM, CP, NFE, TDN, and EE consumptions were higher ($p<0.05$) in T3 compared to those in T0. It was most likely that choline chloride supplementation supplies methyl group to synthesize phosphatidylcholine as a biological membrane component and to support metabolic function of biological membrane (Watson, 2015). According to Casares *et al*. (2019), Phosphatidylcholine is a biological membrane compiler, which is important for maintaining membrane integrity. The resultant of various important functions of choline which are reflected in maintaining the integrity of the membrane will increase the responsiveness of cells to nutrient intake, namely an increase in metabolic rate. Increased metabolic rate requires higher energy support. The increased need for energy stimulated appetite so that the consumption of DM increased in line with the increase in Choline Chloride supplementation at the level of 0.435% DM (T3). The increased consumption of DM would be accompanied by an increase in the consumption of DM components, including protein, NFE, TDN, and EE (Table 1).

Blood lipid status

Blood lipid status of the experimental bulls were evaluated under variables of blood triglyceride (TG), total cholesterol, *Low Density Lipoprotein* (LDL) cholesterol, and *High Density Lipoprotein* (HDL) cholesterol (Table 2).

Triglyceride. There is a relationship between triglycerides and spermatogenesis that appears on the high levels of triglycerides, which can inhibit glucose utilization by Sertoli cells and; thus, affect spermatogenesis (Kim *et al*., 2017). Triglyceride (TG) found in blood comes from absorbed long chain fatty acid of feed that when entering intestine mucosa cell, it is synthesized into TG and transforms into lipoprotein, especially *Very Low Density Lipoprotein* (VLDL), before entering circulation system (Budoff, 2016). The lipoprotein in the circulation system supplies TG to various tissues including lever and extra hepatic tissue (Budoff, 2016). The blood triglyceride may also come from lever through fatty acid esterification produced by adipose tissue mobilization that later is transformed in VLDL and entering the circulation system (Budoff, 2016).

Table 2 shows that the levels of blood TG in the treatment groups T0, T1, T2, and T3 are 15.33, 17.00, 17.67, and 23.67 (mg/dL), respectively. The increased ($p<0.05$) TG level in the blood of T2 and T3 seemed due the increased lipid consumption as well as the increased consumption of feed DM in the respective bulls. The increased TG of the blood plasma was also the result of the increased TG secretion generated from the resistance in lever in using long chain

Table 1. Nutrient consumption

Variables	Treatment				SEM	Significance
	T0	T1	T2	T3		
DM consumption (kg/head/day)	14.32^{b}	14.96^{ab}	16.87^{ab}	17.47^{a}	0.79	p<0.05
CP consumption (kg/ head/day)	1.73^{b}	1.80^{ab}	2.00^{ab}	2.07^{a}	0.24	p<0.05
NFE consumption (kg/head/day)	8.22^{b}	8.62^{ab}	9.82^{ab}	10.20^{a}	0.53	p<0.05
TDN consumption (kg/head/day)	9.69^{b}	10.12^{ab}	11.40^{ab}	11.80^{a}	0.49	p<0.05
EE consumption (kg/head/day)	0.51^{b}	0.52^{ab}	0.55^{ab}	0.56^{a}	0.01	p<0.05

^{a,b,c,d} Different superscript on the same row shows the significant differences ($p<0.05$);
T0 (control) = ration (grass + commercial concentrate) + protected protein suppl. “SOYXYL”;
T1 = control + choline chloride 0.145% DM concentrate/head/day;
T2 = control + choline chloride 0.290% DM concentrate/head/day;
T3 = control + choline chloride 0.435% DM concentrate/head/day;
DM= Dry Matter; CP=Crude Protein; NFA=Nitrogen Free Extract; TDN=Total Digestible Nutrients; EE= Ether extract.

Table 2. Blood lipid and protein status of experimental bulls

Variables	Treatment				SEM	Significance
	T0	T1	T2	T3		
Triglyceride (mg/dL)	15.33^{c}	17.00b^{c}	17.67^{b}	23.67^{a}	0.71	p<0.05
Total cholesterol (mg/dL)	85.67^{b}	88.67^{b}	91.33^{b}	106.67^{a}	3.24	p<0.05
HDL cholesterol (mg/dL)	60.33^{b}	62.67^{b}	63.67^{b}	75.33^{a}	2.11	p<0.05
LDL cholesterol (mg/dL)	32.00^{a}	28.33^{ab}	25.67^{b}	24.67^{b}	1.19	p<0.05
Blood Plasma protein (g/dL)	6.44^{c}	7.82^{b}	8.16^{b}	8.57^{a}	0.17	p<0.05
Blood Plasma albumin (g/dL)	3.17^{b}	3.28^{b}	3.31^{b}	3.56^{a}	0.05	p<0.05
Blood Plasma globulin (g/dL)	4.26^{b}	4.76^{ab}	4.85^{ab}	5.19^{a}	0.32	p<0.05

^{a,b,c,d} Different superscript on the same row shows the significant differences ($p<0.05$).
T0 (control) = ration (grass + commercial concentrate) + protected protein suppl. “SOYXYL”;
T1 = control + choline chloride 0.145% DM concentrate/head/day;
T2 = control + choline chloride 0.290% DM concentrate/head/day;
T3 = control + choline chloride 0.435% DM concentrate/head/day.

fatty acid resulted from mobilization of TG tissue, mainly, adipose tissue, that enter blood circulation as VLDL component. The increased lipid mobilization takes place, as the increased metabolic level occurs (Budoff, 2016; Airaodion *et al*., 2019), and the existence of the increased metabolic level is caused by an increased intracellular enzyme activity stimulated by cAMP from ATP through a catalysis by cyclase adrenal enzyme (Airaodion *et al*., 2019). The increased activity of the enzyme by second messenger that presumably increase its synthesis in line with the increase of the availability of methyl supplemented by choline chloride to form phosphatidylcholine which is part of the second messenger precursor in bio membrane (Airaodion *et al*., 2019).

Total Cholesterol. The level of total cholesterol of blood plasma in the treatment group T0, T1, T2, and T3 were 85.67, 88.67, 91.33, and 106.67 mg/dL, respectively. The supplementation of choline chloride up to 0.290% DM at bulls supplemented with protected protein (3% from concentrate) tended to increase the total cholesterol in total blood plasma. The increase of the total cholesterol was significantly difference ($p<0.05$) occurred in choline chloride supplementation at the level of 0.435%. The increased consumption of lipid (Ether extract) was in line with proportion to the increase of the level of choline chloride supplementation (Table 1). According to Gallier and Singh (2012), the lipid transported in the transportation system happens through lipoprotein formation in which free and esterification lipid exists. The formation of the lipoprotein increases corresponding to the increased lipid absorption as a result of the increased nutrient consumption. The increased lipoprotein synthesis followed by the increased cholesterol synthesis causes the total level of cholesterol increases. The choline chloride plays a role in the synthesis of lipoproteins for lipid transport. In this case choline chloride supplies the methyl group to the synthesis of phosphatidylcholine which is the main part of lipoprotein.

HDL Cholesterol. The level of HDL cholesterol of blood plasma at the treatment group T0, T1, T2, and T3 were 60.33, 62.67, 63.67, and 75.33 mg/dL, respectively (Table 2). The level of the HDL cholesterol increased, as the supplementation of choline chloride given was up to 0.290%. The significantly increased ($p<0.05$) level of HDL cholesterol was found in the treatment group supplemented by 0.435% choline chloride, which was 75.33 mg/dL.

According to Ouimet *et al*. (2019), HDL synthesized either in liver or in intestine functions to support absorbed lipid transportation. The increased lipid absorption is followed by the increased HDL synthesis to transport nutrient; as a result, the level of HDL increases. The HDL synthesis is also supported by sufficient protein availability, in this case, it was taken from the protected protein supplementation (SOYXYL) containing in the ration.

LDL Cholesterol. Table 2 shows that the level of LDL cholesterol in the treatment group T0, T1, T2, and T3 are 32.00, 28.33, 25.67, and 24.67, respectively. The level of LDL cholesterol tended to decrease at the level of 0.145% supplementation of choline chloride. The significant decrease ($p<0.05$) was found at the supplementation of choline chloride at the level of 0.290% and 0.435%. The decreased level of LDL cholesterol took place in accordance with the increased level of HDL cholesterol. According to Ouimet *et al*. (2019), HDL facilitates blood cholesterol taken from extrahepatic tissue and other lipoprotein to be supplied to lever and metabolized. This mechanism was likely to decrease LDL cholesterol level while HDL cholesterol level increased.

Blood protein status

Blood protein status could be evaluated on the basis of the protein level of total plasma and essential protein in blood plasma; such as, albumin and globulin.

Blood Plasma Protein. The data of the protein level of the blood plasma show that the level of plasma protein at the treatment group T0, T1, T2, and T3 were 6.44, 7.82, 8.16, and 8.57 g/dL, respectively (Table 2). The choline chloride supplementation at the experimental bulls increased the level of blood plasma protein ($p<0.05$). The highest level of blood plasma protein was found to be in the treatment group T3, which was supplemented with 0.435% choline chloride. This phenomenon might happen, as there was an increased in protein bio synthesis at the treatment group supplemented with choline chloride. Furthermore, choline chloride can also function as methyl donor to synthesize amino acid; such as, methionine, carnitine, and phosphatidylcholine (Harvey and Ferrier, 2011). They further explained that as methionine is a limiting essential amino acid, the increase of its availability will increase the efficiency of protein biosynthesis. Carnitine is an amino acid that might function to facilitate the entering of fatty acid into mitochondria to be oxidized so that the availability of the energy will be engaged for supporting protein biosynthesis. Meanwhile, the phosphatidylcholine is the second messenger precursor that stimulates adenylyl enzyme of bio membrane cyclase to form cAMP from ATP, so enzymes catalyzed intracellular metabolism is activated. The increased level of the intracellular metabolism affects the increased protein biosynthesis that later appears in the increased level of the blood plasma protein.

Blood Plasma Albumin. The analysis of the level of blood plasma albumin shows that the level of blood plasma albumin at the treatment group T0, T1, T2, and T3 were 3.17, 3.28, 3.31, and 3.56 g/dL, respectively (Table 2). The level of blood plasma albumin tended to increase in the supplementation of choline chloride at the level of

up to 0.290%. The significantly increased level of blood plasma albumin ($p<0.05$) was found in treatment group supplemented by 0.435% choline chloride. The increased level of the blood plasma albumin took place, as there is an increase in protein biosynthesis following the increased level of the metabolic (Constable *et al*., 2019) that was resulted by choline chloride supplementation taken from the availability of essential amino acid from SOYXYL; therefore, the efficiency of the protein biosynthesis increased. The increased level of blood plasma albumin may also take place in line with the increased need of that compound to transport lipid acid resulted from adipose tissue mobilization to lever and other extra hepatic tissue (Constable *et al*., 2019). The level of blood plasma albumin at four treatment groups was in normal range, that is 2.1-3.6 g/dL (Radostits *et al*., 2007).

Blood Plasma Globulin.– The level of blood plasma globulin of bulls at treatment group T0, T1, T2, and T3 is 4.26, 4.76, 4.85, and 5.19 g/dL, respectively (Table 2). The level of blood plasma globulin tended to increase as a result of choline chloride supplementation given up to the level of 0.290%. The significantly increased level of globulin ($p<0.05$) was found in the choline chloride supplementation at level of 0.435%. This explained that the choline chloride given to the bulls fed with SOYXYL caused the increased protein biosynthesis as well as the increased level of blood plasma globulin. The increased level of blood plasma globulin was presumably occurred as a result of the increased need of protein to transport steroid hormone (in this case testosterone) that its production increased in bulls fed with SOYXYL due to choline chloride supplementation (Table 2). The level of blood plasma globulin of the experimental bulls was in normal range, which is 2.9 – 4.9 g/dL (Radostits *et al*., 2007).

Reproduction performance

Reproduction performance of the experimental bulls was reflected on the variables of testosterone hormone concentration, sperm concentration, and sperm motility (Table 3).

Testosterone Hormone Concentration.– Testosterone hormone concentration of blood plasma on T0, T1, T2, and T3 is 4.6600, 5.4767, 7.220, and 9.070 ng/mL, respectively (Table 3). The supplementation of choline chloride to the experimental bulls supplemented with 3% of protein bypass significantly increased ($p<0.05$) the concentration of testosterone hormone. The hormone concentration of blood plasma testosterone was higher following the increased level of choline chloride supplementation (up to 0.435% from DM concentrate).

According to Lauro *et al*. (2016), the increased level of choline chloride supplementation intensifies blood cholesterol level leading to the increased concentration of testosterone hormone. Cholesterol is a precursor compound of steroid hormones, such as testosterone hormone. Meanwhile, the increased biosynthesis of steroid hormone is supported by the availability of enzyme catalyzing the process of the biosynthesis hormone. In this case, the availability of the enzyme causing the increased protein level of blood plasma was caused by the increased level of choline chloride supplementation up to 0.435% from DM concentrate.

According to Wang *et al*. (2017), testosterone hormone is produced by Leidyc cells as the spot of the main steroidogenesis in the testes. The increased production of testosterone hormone as a result of choline chloride supplementation to bulls supplemented by protected protein was also taken place at the cellular level, which was the increased proliferation of the Leidyc cells.

Sperm Concentration. The sperm concentration on T0, T1, T2, and T3 is 1006.74, 1061.19, 1151.59, and 1270.41 million/mL, respectively (Table 3). Choline chloride given to the experimental bulls supplemented with up to 0.435% from DM concentrate proved to increase sperm concentration ($p<0.05$).

When choline chloride interacts with folate acids in using methionine for biologic and biosynthetic, it is an essentially metabolic base of cells proliferation in various tissues, including spermatogenesis taken place in *tubuli seminiferi* in testes through both meiosis and mitosis germ cells (Niculescu and Zeisel, 2002). Thus, the essential function of the nutrients increased spermatogenesis while choline chloride was given to the bulls supplemented with protected protein as reflected in the increased sperm concentration. Meanwhile, testosterone hormone increases protein anabolism in proliferation of spermatozoa cells in the spermatogenesis process (Birzniece *et al*., 2011). Therefore, choline chloride given to the bulls supplemented with protected

Table 3. Reproduction and body weight gain performance of experimental bulls

Variables	Treatment				SEM	Significance
	T0	T1	T2	T3		
Testosterone hormone concentration (ng/mL)	4.6600^{c}	5.4767^{c}	7.220^{b}	9.070^{a}	1.83	p<0.05
Sperm concentration (million/mL)	1006.74^{d}	1061.19^{c}	1151.59^{b}	1270.41^{a}	16.12	p<0.05
Sperm motility (%)	36.00^{c}	40.67^{bc}	45.00^{b}	70.00^{a}	2.09	p<0.05
Body weight gain (kg/day)	0.37	0.43	0.45	0.60	0.09	p>0.05

^{a,b,c,d} Different superscript on the same row shows the significant differences ($p<0.05$).
T0 (control) = ration (grass + commercial concentrate) + protected protein suppl. "SOYXYL";
T1 = control + choline chloride 0.145% DM concentrate/head/day;
T2 = control + choline chloride 0.290% DM concentrate/head/day;
T3 = control + choline chloride 0.435% DM concentrate/head/day.

protein increased the concentration of the testosterone hormone (Table 3).

Sperm Motility. The sperm motility on T0, T1, T2, and T3 are 36.00, 40.67, 45.00, and 70%, respectively (Table 3). Cheach and Yang (2011) stated that cAMP level determines the sperm motility, as this compound is formed from ATP through enzymatic reaction catalyzed by cyclase adenylyl enzyme. The adenylyl cyclase enzyme is activated by the second messenger formed from phosphatidylcholine. The supplementation of choline chloride contributes to phosphatidylcholine biosynthesizing as the second messenger to activate adenylyl cyclase. This mechanism underlined the fact that the sperm motility significantly increased ($p<0.05$) as a result of choline chloride supplementation at the level of 0.290% and 0.435% from DM concentrate.

Body Weight Gain. The body weight gain (BWG) of bulls supplemented with protected protein and choline chloride was not significantly increased compared to those without being given choline chloride supplementation. The BWG on T0, T1, T2, and T3 is 0.37, 0.43, 0.45, and 0.60 kg/day, respectively (Table 3).

The choline chloride given to the bulls supplemented with protected protein increases bulls' metabolic level; increased energy availability and protein synthesis used for supporting production of reproduction hormone and spermatogenesis (Niculescu and Zeisel, 2002; Birzniece *et al*., 2011). The phenomenon of the increased level of testosterone hormone and sperm concentration was identified to happen in the bulls supplemented with choline chloride. The production of the testosterone hormone and sperm concentration was even higher as the choline chloride given were increased up to 0.435% from DM concentrate. As a result, there was no significant difference on the BWG among treatment groups, although the consumption of DM feed in the group supplemented with choline chloride was higher than that of T0, and the DM feed consumption increased in line with the increased of the supplementation level of the choline chloride. The findings support previous study which reported that rumen-protected choline (RPC) supplements cannot affect growth performance in Holstein young bulls, but lipid metabolism may be affected by RPC (Hajilou *et al*., 2014).

There was no significant difference on BWG although the DM feed consumption was increased suggested that the increased consumption of DM feed did not increased fat deposition in the adipose tissues. In fact, the metabolic status support reproduction performance leading to bulls' fertility (Rato *et al*., 2012).

Conclusion

The supplementation of choline chloride up to 0.435% from dry matter concentrate to bulls supplemented with protected protein improves hematologic status for reproduction purposes that can be identified from the increased concentration of testosterone hormone and sperm without adding the body weight gain of the bulls.

Acknowledgment

The authors would like to extend appreciation to the Rector and the Head of the LPPM (community development and research institute) of Diponegoro University for providing fund (Project No. 385-38/UN7.P4.3/PP/2019, Fiscal Year 2019) and approving the study. We would also like to extend our gratitude to the Dean of Faculty of Animal and Agricultural Sciences, Diponegoro University for approving the research proposal and providing facilities including laboratory to conduct the research. Our appreciation also goes to students who carried out the field study.

References

Airaodion, A. I., U. Ogbuagu, E. O. Ogbuagu, A. P. Oloruntoba, A. P. Agunbiade, E. O. Airaodion, I. P. Mokelu, and S. C. Ekeh. 2019. Mechanisms for controlling the synthesis of Lipids – Review. Int. J. Res. 6: 123-135.

Birzniece, V., U. J. Meinhardt, M. A. Umpleby, D. J. Handelsman, and K. K. Y. Ho. 2011. Interaction between testosterone and growth hormone on whole-body protein anabolism occurs in the liver. J. Clin. Endocrinol. Metab. 96: 1060–1067. https://doi.org/10.1210/jc.2010-2521.

Budoff, M. 2016. Triglicerides and triglyceride-rich lipoprotein in causal oathway of cardio vascular disease. Am. J. Cardiol. 118: 138-145. https://doi.org/10.1016/j.amjcard.2016.04.004

Casares, D., P. V. Escribá, and C. A. Rosselló. 2019. Membrane lipid composition: Effect on membrane and organelle structure, function and compartementalization and therapeutic avenues. Int. J. Mol. Sci. 20(9): 2167-2175. https://doi.org/10.3390/ijms20092167

Chandler, T. L. and H. M. White. 2017. Choline and methionine differentially alter methyl carbon metabolism in bovine neonatal hepatocytes. PLoS One 12: e0171080. https://dx.doi.org/10.1371%2Fjournal.pone.0171080

Chaudhari, K. I., D. C. Prajapati, P. M. Lunagariya, K. K. Sorathiya, S. N. Patel, R. P. Patel, and A. L. Nayak. 2017. An importance of choline chloride for poultry and cattle: An overview. Int. J. Sci. Environ. Technol. 6: 2804-2810. http://www.ijset.net/journal/1895.pdf

Cheach, J. and W. Yang. 2011. Functions ofessential nutrition for high quality

spermatogenesis. Advances in Bioscience and Biotechnology. 2: 182-197. Doi:10.4236/abb. http://www.SciRP.org/journal/ab/

Constable, P., F. M. Trefz, and H. Stampfly. 2019. Effects oh pH and the plasma or serum concentrations, l-lactate, and albumin on the plasma ionized calcium concentration in calves. J. Vet. Int. Med. 33: 1822-1832. https://doi.org/10.1111/jvim.15509

Gallier, S. and H. Singh. 2012. The physical and chemical structure of lipids in relation to digestion and absorption. Lipid Technology. 24: 271-273. https://doi.org/10.1002/lite.201200240

Gorissen, S. H. M., J. J. R. Crombag, J. M. G. Senden, W. A. H. Waterval, J. Bierau, L. B. Verdijk, and L. J. C. V. Loon. 2018. Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids 50: 1685–1695. https://doi.org/10.1007/s00726-018-2640-5

Grummer, R. 2016. Choline: A limiting nutrient for transition dairy cows. Department of Dairy Science University of Wisconsin, Madison. file:///D:/PUBLIKASI%20BIB%20APRIL%20%202020/GRUMMER%202016.%20SOUTHWEST-NUTRITION-AND-MANAGMENT-CONFERENCE-2016.pdf

Hajilou, M., M. Dehghan-Banadaky, A. Zali, and K. Rezayazdi. 2014. The effects of dietary L-carnitine and rumen-protected choline on growth performance, carcass characteristics and blood and rumen metabolites of Holstein Young bulls. J. Appl. Anim. Res. 42: 89-96. http://dx.doi.org/10.1080/09712119.2013.822807

Harvey, R. A. and D. R. Ferrier. 2011. Illustrated Reviews: Biochemistry. 5th edn. Lipponcott Williams & Wilkins, Philadelphia. p. 520.

Kim, N., H. Nakamura, H. Masaki, K. Kumasawa, K. Hirano, and T. Kimura. 2017. Effect of lipid metabolism on male fertility. Biochemical and Biophysical Research Communications. 485: 686-692. https://doi.org/10.1016/j.bbrc.2017.02.103

Lagace, T. A. 2016. Phosphatidylcholine: Greasing the Cholesterol Transport Machinery. Lipid Insights 8: 65–73. https://doi.org/10.4137%2FLPI.S31746

Lauro, F. V., D. C. Francisco, G. C. Elodia, R. N. Marcela, P. G. Eduardo, L. R. Maria, and C. L. Abelardo. 2016. Design and synthesis three steroid-tetraone derivatoives using testosterone as chemical tool. Letters in Organic Chemistry. 13: 591-603. DOI **:** 10.2174/1570178613666160824143943

Niculescu, N. D. and S. H. Zeisel. 2002. Diet, methyl donor and DNA methylation: Unteractions between dietary folate, methionine and choline. J. Nutr. 132: 2333-2335. https://doi.org/10.1093/jn/132.8.2333S

Ouimet, M., T. J. Barret, and E. A. Fisher. 2019. HDL and reverse cholesterol transport, basic mechanisms and their roles in vascular health and disease. Circulation Research. 124: 1505-1518. https://doi.org/10.1161/CIRCRESAHA.119.312617

Prasetiyono, B. W. H. E., Suryahadi, T. Toharmat, and R. Syarief. 2007. Strategi suplementasi protein ransum sapi potong berbasis jerami dan dedak padi. Media Peternakan 30: 207-217.

Prasetiyono, B. W. H. E., Widiyanto, Y. S. Ondho, A. Subrata, and H. S. Widodo. 2020. Effect of SOYXYL as a Dietary Protected Protein Supplement on the Reproductive Performance of Simmental Bulls. Animal Production. 22: 1-8.

Radostits, O. M., C. C. Gay, K. W Hinchcliff, and P. D. Constable. 2007. Veterinary medicine: A textbook of the disease of cattle, sheep, goats, pigs and horses. 10th edn. Elsevier Healts Sciences, Philadelphia, PA, USA.

Rato, L., M. G. Alves, S. Socorro, A. I. Duarte, J. E. Cavaco, and P. F. Oliveira. 2012. Metabolic regulation is important for spermatogenesis. Nat. Rev. Urol. 9: 330–338. https://doi.org/10.1038/nrurol.2012.77

Sachidhanandam, M., S. N. Singh, A. K. Salhan, and U. S. Ray. 2010. Evaluation of plasma hormone concentrations using enzyme-immunoassay/enzyme-linked immunosorbent assay in healthy Indian men: effect of ethnicity. Indian J. Clin. Biochem. 25: 153-157.

SAS. 2009. SAS User's Guide. SAS Institute Inc, SAS Campus Drive, Cary, NC 27513.

Steel, R. G. D., J. H. Torrie, and D. A. Dickey. 1996. Principles and Procedures of Statistic: A Biometrical Approach. McGraw-Hill College.

Vijayalakshmy, K., M. Virmani, D. Kumar, P. Kumar, and H. Rahman. 2018. Different Methods of Assessing Semen Quality. Indian Farmer 5: 1383-1387.

Wang, Y., F. Chen, L. Ye, B. Zirkin, and H. Chen. 2017. Steroidogenesis in Leydig cells: effects of aging and environmental factors. Reproduction. 154: R111-R122. https://doi.org/10.1530/REP-17-0064.

Watson, H. 2015. Biological membranes. Essays Biochem. 59: 43-69: https://doi.org/10.1042/bse0590043.