Meta-Analysis of the Effects of Rumen Protected Choline Supplementation on Milk Production and Reproduction in Dairy Cows

Yusuf Wili Prihantoro, Rudy Hartanto, Daud Samsudewa*

Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia
***Corresponding author's email: daudreproduksi@gmail.com**

Abstract. This study seeks to comprehensively assess the impact of Rumen Protected Choline (RPC) supplementation on key parameters in dairy cows, including dry matter intake, milk yield, milk fat and protein composition, first estrus, open days, and Service per Conception (S/C). This research utilizes a quantitative meta-analysis methodology to systematically evaluate and synthesize data. The treatment used is supplementation Rumen Protected Choline and the basal diet for control. Observed parameters include dry matter intake (DMI), milk yield (MY), milk fat content (MFC), milk protein content (MPC), service per conception (S/C), first estrus (FE), and open days (OD). The study went through five stages, namely database preparation, meta-analysis implementation, heterogeneity testing, summary effect testing, and publication bias measurement. Tools used include PDF software for filtering journals and data processing applications such as Microsoft Excel, OpenMEE Software, and Review Manager 5.4 for data interpretation. Based on the results of the meta-analysis, it is found that RPC supplementation significantly affects ($p<0.05$) the increase in milk production, milk protein content, and milk fat content. It is also capable of reducing S/C and open days, but it does not affect dry matter intake and first estrus. The findings of this study, derived from a meta-analysis, indicate that while RPC supplementation has no impact on feed intake, it significantly influences reproductive parameters.

Keywords: Production, Reproduction, Rumen protected choline

Abstrak. Penelitian ini bertujuan untuk menilai secara komprehensif dampak suplementasi *Rumen Protected Choline* (RPC) terhadap parameter utama pada sapi perah, termasuk asupan bahan kering, produksi susu, komposisi lemak dan protein susu, estrus pertama, hari berahi, dan *Service per Conception* (S/C). Penelitian ini menggunakan metodologi meta-analisis kuantitatif untuk mengevaluasi dan mensintesis data secara sistematis. Perlakuan yang digunakan adalah suplementasi *Rumen Protected Choline* dan diet basal sebagai kontrol. Parameter yang diamati meliputi asupan bahan kering (DMI), produksi susu (MY), kandungan lemak susu (MFC), kandungan protein susu (MPC), *service per conception* (S/C), estrus pertama (FE), dan hari berahi (OD). Penelitian ini melalui lima tahap, yaitu persiapan basis data, implementasi meta-analisis, pengujian heterogenitas, pengujian efek ringkasan, dan pengukuran bias publikasi. Alat bantu yang digunakan antara lain perangkat lunak PDF untuk menyaring jurnal dan aplikasi pengolah data seperti Microsoft Excel, OpenMEE Software, dan Review Manager 5.4 untuk interpretasi data. Berdasarkan hasil meta-analisis, ditemukan bahwa suplementasi RPC secara signifikan memengaruhi ($p<0,05$) peningkatan produksi susu, kandungan protein susu, dan kandungan lemak susu. Suplementasi RPC juga mampu mengurangi S/C dan hari berahi, tetapi tidak memengaruhi asupan bahan kering dan estrus pertama. Temuan penelitian ini, yang berasal dari meta-analisis, menunjukkan bahwa meskipun suplementasi RPC tidak berdampak pada asupan pakan, namun secara signifikan memengaruhi parameter reproduksi.

Kata kunci: produksi, reproduksi, kolin yang dilindungi rumen

Introduction

Dairy cattle play a crucial role in Indonesia's livestock industry. However, the current population remains inadequate to meet national milk consumption demands, highlighting the need for herd expansion. A major challenge is low reproductive performance due to postpartum stress. Postpartum stress negatively impacts nutrient intake, affecting both production and reproduction. Nutrients consumed through feed are redirected to stabilize the body condition, resulting in Negative Energy Balance (NEB) (Grummer, 2008). The body's response to NEB in dairy cattle involves mobilizing energy reserves, producing ketones to meet requirements. Excessive ketone production can lead to liver fat accumulation (McArt et al., 2015).

Choline serves as a key transporter of liver fat and is essential for the synthesis of phosphatidylcholine, a vital component of very-low-density lipoproteins (VLDL) (Ardalan et al., 2009) and for protein synthesis. Thus, supplementation benefits stressed cows by supporting gluconeogenesis and protein synthesis, aiding milk production and reproduction.

Previous studies have reported mixed results on the effectiveness of RPC supplementation. Some report positive effects on milk production and reproduction (Zahra et al., 2006; Zom et al., 2011), while others on the contrary (Morrison et al., 2018). Thus, a meta-analysis is necessary to provide more accurate insights into the effects of RPC supplementation in dairy cows.

This study aims to assess the impact of RPC supplementation on dry matter intake, milk yield, milk fat and protein content, first estrus, open days, and Service per Conception (S/C) in dairy cows.

Materials and Methods

This meta-analysis is based on data obtained from internationally recognized and accredited journals. A literature search was conducted, yielding 310 journals in hopes of finding data related to each aspect of the study (Dry Matter Intake, MY, MFC, MPC, S/C, Calving Interval, and Empty Days). Among these, 14 relevant journals were identified and included in the analysis. The study utilized PDF software for journal screening and data processing applications such as Microsoft Excel, OpenMEE Software, and Review Manager 5.4 for data interpretation.

Data Compilation

This meta-analysis investigated the impact of RPC supplementation as an experimental treatment. This was done with the intention of observing the effects of RPC supplementation o DMI, MY, MFC, MPC, calving interval, and empty days, comparing results with the control group (without RPM supplementation) in Table 1.

Table 1. Database of journal on the effect of rumen-protected choline supplementation on the production and reproduction of dairy cows.

No	Author	Year	Parameter	Treatment	Duration
1	Amrutkar et al.,	2015	S/C, FE	RPC	160 d
2	Acharya et al.,	2020	S/C, FE	RPC	90 d
3	Ardalan et al.,	2009	S/C, OD, FE	RPC	112 d
4	Çetin et al.,	2018	MY	RPC	81 d
5	Chavda et al.,	2022	S/C, FE	RPC	90 d
6	Davidson et al.,	2008	MY, DMI, MPC, MFC	RPC	112 d
7	Elek et al.,	2008	MY, DMI, MPC, MFC	RPC	81 d
8	Guretzky et al.,	2006	MY, MPC, MFC	RPC	42 d
9	Hartwell et al.,	2000	MY, DMI, MPC, MFC	RPC	148 d
10	Lunagariya et al.,	2023	S/C	RPC	121 d
11	Mečionytė et al.,	2022	S/C	RPC	84 d
12	Piepenbrink et al.,	2003	MY, DMI	RPC	84 d
13	Pirestani and Aghakhani	2018	S/C, FE	RPC	35 d
14	Potts et al.,	2020	MY, DMI, MPC, MFC	RPC	55 d
15	Toledo et al.,	2017	MY, MPC, MFC	RPC	96 d
16	Xu et al.,	2006	MY, DMI	RPC	28 d
17	Zahra et al.,	2006	MY	RPC	49 d
18	Zhou et al.,	2016	MY, DMI, MPC, MFC	RPC	51 d

Note: S/C (Service per conception), FE (First estrus), OD (Open days), MY (Milk yield), DMI (Dry matter intake), MPC (Milk protein content), MFC (Milk fat content)

The Implementation Stage of Meta-Analysis

The study commenced with data compilation, which involved selecting relevant studies for inclusion in the meta-analysis. The meta-analysis utilized secondary data obtained from published and publicly available journals. Studies included in the meta-analysis underwent a selection process based on inclusion and exclusion criteria. Studies were selected from English-language international journals published between 2000 and 2023. These journals were identified through searches on databases such as SCOPUS, Science Direct, Wiley, PubMed, and Google Scholar, resulting in a total of 310 journals. The identified journals then underwent further selection based on inclusion criteria, resulting in a final selection of 14 journals used in the meta-analysis. The data for this study were exclusively obtained from journals published between 2000 and 2023, using keywords such as Rumen Protected Choline, dry matter intake, milk fat content, milk protein content, milk yield, service per conception, open days, and calving interval to ensure relevant literature selection.

Figure 1 presents the results of the article selection process, highlighting the inclusion of journals based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) methodology, generated using Review Manager 5.4.1. The meta-analysis results are interpreted through effect size values, followed by publication bias assessment using a funnel plot and the fail-safe N method.

Results and Discussion

Dry Matter Intake

An analysis of 28 studies examined the effects of RPC on dry matter intake. The findings, including publication bias assessment, forest plot evaluation, and heterogeneity analysis, are summarized in Table 2. The findings indicate that RPC supplementation had no significant effect on dry matter intake ($p \geq 0.05$) compared to the control. However, publication bias was detected in studies with RPC treatment, as the Fail-Safe N value exceeded the Limit Fail-Safe N value.

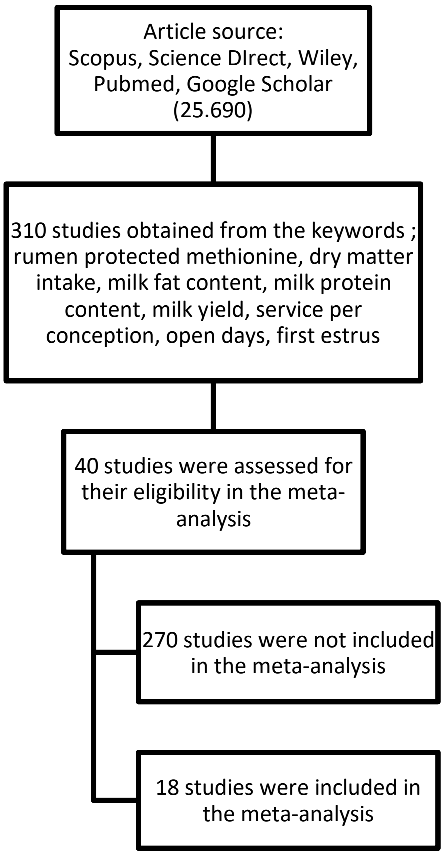

Figure 1. Article Selection and Evaluation using PRISMA Method

Dry matter intake (DMI) plays a crucial role in providing the necessary nutrients for growth, production, and reproduction. Utomo and Miranti (2010) stated that the dry matter content in feed is essential for meeting these nutritional requirements. Several factors influence DMI, including animal health status and environmental conditions. A deficiency in dry matter intake will affect the BCS of dairy cows, subsequently altering hormone secretion, particularly substrate hormones derived from fat and energy reserves (Afif et al., 2023). During the transition period, an energy imbalance known as Negative Energy Balance (NEB) often occurs. NEB happens when the energy consumed by the body is not balanced with the energy used for production and basic needs (Baumgard et al., 2006). Zhou et al. (2016) stated that animals experiencing NEB have an increased potential for ketosis.

Table 2. The results of meta-analysis research on the effect of rumen-protected choline supplementation in dairy cows on Dry Matter Intake (DMI), Milk Yield (MY), Milk Protein Content (MPC), Milk Fat Content (MFC), Service per Conception (S/C), Open Days (OD), and Calving Interval (CI).

Parameter	Treatment[a]		Publication bias		Forest Plot		Het
	RPC	Control	Fail-Safe N Value	Fail-Safe N Limit	Estimate	P-Value	Het P-value
DMI (kg)	16.4 ± 4.23	16.1 ± 4.15	0	150	0.131	0.124	0.461
MY (kg)	35.6 ± 1.82	33.9 ± 1.82	117	110	0.428	0.008	<0.001
MPC (kg)	1.11 ± 0.16	1.07 ± 0.17	45	80	0.410	0.027	0.004
MFC (kg)	1.41 ± 0.26	1.37 ± 0.24	22	80	0.313	0.027	0.135
S/C (times)	2.05 ± 0.26	2.50 ±0.27	35	45	-0.606	0.006	0.053
OD (days)	107 ± 20.7	133 ± 30.5	39	35	-1.287	0.017	<0.001
FE (days)	66.7 ±15.9	72.7 ± 19.0	0	35	-0.438	0.375	<0.001

Note: DMI (Dry matter intake), MY (Milk yield), MPC (Milk protein content), MFC (Milk fat content), S/C (Service per conception), OD (Open days), FE (First estrus)

Incorporating RPC into the diet affects nutrient availability, enhancing metabolism and supporting milk synthesis. Nutrient availability plays a role in milk synthesis; higher intake results in increased milk production, and nutrient-rich feed such as choline, used for fatty acid transport in the liver, can yield good milk production results (Piepenbrink and Overton, 2003). Sun et al. (2016) stated that RPC supplementation conserves amino acids for milk production, enabling nutrients to be utilized more efficiently to enhance the animal's metabolic health.

Enhancing the animal's metabolic condition helps alleviate stress, promoting overall well-being as metabolic processes stabilize. Elsaadawy et al. (2022) reported that choline supplementation improved energy balance, leading to increased feed intake by influencing feed consumption control via the nervous system. However, RPC does not directly affect feed intake, as its primary function is facilitating fatty acid transport in the liver. According to Rahmani et al. (2014), choline protected in the rumen (RPC) is given to dairy cows to increase choline supply to the small intestine, aiming to improve milk yield or its components, or reduce the development of fatty liver by increasing fat removal from the liver.

Milk Yield

An analysis of 20 studies investigated the effects of RPC on milk yield. The results, including publication bias assessment, forest plot evaluation, and heterogeneity analysis, are detailed in Table 2. The results indicate that RPC supplementation significantly increased milk production compared to the control ($p<0.05$). The estimated effect size for RPC is 0.428, categorized as moderate. Publication bias testing suggests no bias in the meta-analysis results.

Milk yield is directly influenced by feed intake, as the nutrients derived from consumed feed serve as the building blocks for milk production. Tyler and Ensminger (2006) emphasized that milk biosynthesis relies on essential precursors and substrates obtained through digestion. In ruminant animals, glucose is produced in the liver through gluconeogenesis using volatile fatty acids (VFA, the main substrate), lactate, and amino acids as fuel, as well as through direct absorption from the intestine. With sufficient glucose supply, the mobilization of fat from adipose tissue for βoxidation can decrease, saving amino acids for glycogen formation. RPC can reduce plasma cholesterol levels (Total Cholesterol and HDL-C) and increase glucose levels, leading to increased lactose levels (Sun et al., 2016). According to Alhusein (2018), an increase in lactose content in milk can improve milk production due to the role of lactose as an osmoregulator in the mammary gland.

Studies on the impact of RPC supplementation on milk production have produced inconsistent findings. Zhou et al. (2016) reported that variations in milk yield among RPC-treated animals are influenced by factors such as the duration of supplementation, dosage levels, and the overall health status of the cows. Choline contributes to increased milk production by facilitating fatty acid absorption in the liver. Cetin et al. (2018) emphasized choline’s critical role in milk synthesis and component production, as it supports fatty acid transport in the liver and serves as a precursor for milk formation.

Milk Protein Content

An analysis of 14 studies evaluated the impact of RPC supplementation on milk protein content. Publication bias testing, forest plot, and heterogeneity analysis are presented in Table 2. The results indicate that RPC supplementation significantly increases milk protein content ($p<0.05$). The estimated effect size for RPC is 0.410, categorized as moderate. However, publication bias was detected in the RPC treatment, as the Fail-Safe N value exceeded the Limit Fail-Safe N value.

Milk protein is formed from nutrients that are absorbed into the bloodstream during digestion and subsequently utilized in the milk synthesis process. Prihatminingsih et al. (2015) stated that milk protein content depends on the protein composition of the feed, as an adequate supply of amino acids in the blood serves as precursors for milk production. An increase in RPC administration to milk protein is due to the utilization of fatty acids in the liver, and RPC administration provides an increased amount of nutrients that can be used as milk precursors. Additionally, choline supplementation has been proven to convserve methionine, an amino acid containing sulfur, for milk protein synthesis (Sun et al., 2016).

Milk Fat Content

An analysis of 14 studies examined the effects of RPC supplementation on milk fat content. The findings, including publication bias assessment, forest plot evaluation, and heterogeneity analysis, are summarized in Table 2. The findings indicate that RPC supplementation significantly increases milk fat content ($p<0.05$). The estimated effect size for RPC is 0.313, categorized as moderate. However, publication bias was detected, as the Fail-Safe N value exceeded the Limit Fail-Safe N value.

Milk fat content is influenced by both the quality of feed and the physiological condition of lactating cows. Arifin (2011) stated that factors such as feed intake, climate conditions, cattle age, and management practices play a crucial role in determining milk fat levels. The observed increase in milk fat content following RPC supplementation suggests that choline enhances fatty acid transport. Elek et al. (2008) stated that choline plays a crucial role in facilitating fatty acid transport in the blood, thereby increasing the availability of fat for milk fat synthesis. Furthermore, Zhou et al. (2016) suggested that choline supplementation is particularly beneficial for cattle with compromised liver health, as it improves liver fat metabolism, enabling the body to utilize liver fat as a precursor for milk production.

Service per Conception

An analysis of seven studies investigated the effects of RPC supplementation on Service per Conception (S/C). The results, including publication bias assessment, forest plot evaluation, and heterogeneity analysis, are presented in Table 2. The findings indicate that RPC supplementation significantly affects S/C ($p<0.05$). However, publication bias was detected, as the Fail-Safe N value exceeded the Limit Fail-Safe N value.

Reproductive ability in animals is strongly influenced by their overall health, with Negative Energy Balance (NEB) significantly reducing fertility. Budiawan et al. (2015) emphasized that fertility is directly dependent on adequate nutrient intake, as deficiencies can disrupt metabolic functions and negatively impact reproductive performance. S/C in animals is closely related to accuracy in detecting animal estrus. Jaenudin et al. (2018) state that detecting estrus in animals significantly determines the success of animal mating, especially for animals undergoing artificial insemination. Animals experiencing NEB will undergo hormonal changes, often causing a decrease in gland function and a decrease in gonadotropin hormone secretion. Hendrawan et al. (2019) suggest that disruptions in gonadotropin hormone secretion in high-yield dairy cows are frequently caused by inadequate nutrient intake, which fails to support metabolic demands. Severe nutritional deficiencies can result in endocrine disturbances, delaying estrus onset. According to Sutiyono et al. (2017), such disturbances lead to low estrogen production, preventing cows from exhibiting clear estrus signs, a condition known as silent estrus. RPC supplementation has been found to significantly influence S/C by improving metabolic conditions and mitigating NEB, thereby supporting follicular development. According to Pirestani and Aghakhani (2018), choline administration can help animals reduce NEB, ensuring follicle development and livestock fertility are not compromised.

Open Days

A study on the effects of RPC on open days analyzed five studies. The results of publication bias testing, forest plot, and heterogeneity analysis are presented in Table 2. The results suggest that RPC supplementation leads to a significant reduction in open days in dairy cows ($p<0.05$). The estimated effect size for RPC is -1.287, suggesting a strong influence on reducing open days. Publication bias testing suggests no bias in the meta-analysis results, as the Fail-Safe N value exceeded the Limit Fail-Safe N value.

Following calving, dairy cows undergo physiological stress due to nutrient deficiencies, necessitating a recovery period before they are ready for mating and pregnancy. Atabany et al. (2008) emphasized that postpartum cows require sufficient time to regain their reproductive readiness for the next pregnancy. The normal duration of open days in dairy cows ranges from 85 to 115 days after calving (Izquierdo et al., 2008). Open days in dairy cows are influenced by various factors, including physiological conditions, nutrient consumption, and mating management. According to Lanyasunya et al. (2005), open days in animals are closely related to problems with the physiological condition of animals influenced by feed consumption and nutrient deficiencies. Nutrient deficiencies will adversely affect the production and reproduction abilities of cows. Wahyudi et al. (2013) highlight that high-yield dairy cows have increased energy demands, which can delay pregnancy due to elevated prolactin levels suppressing gonadotropin hormone secretion. RPC supplementation has been shown to significantly reduce open days in animals. Amrutkar et al. (2015) found that choline supplementation decreases NEB, leading to improved metabolic function and a decrease in open days.

First Estrus

A study on the effects of RPC on first estrus analyzed five studies. The results of publication bias testing, forest plot, and heterogeneity analysis are presented in Table 2. The results indicate that RPC supplementation has no significant effect on the timing of first estrus in dairy cows (p≥0.05). However, publication bias was observed, as the Fail-Safe N value exceeded the Limit Fail-Safe N value.

Conclusions

The meta-analysis findings indicate that RPC supplementation positively enhances milk production, as well as milk protein and fat content. Furthermore, it significantly decreases the Service per Conception (S/C) ratio and the number of days open in dairy cows. However, RPC supplementation does not affect dry matter intake or the timing of the first estrus.

References

Acharya P, SS Lathwal,R Baithalu,N Patnaik, MR Thul and BMoharana. 2020. Supplementing rumen protected choline with green tea extract improves reproductive performances in transition Karan Fries Cows. Indian Journal of Animal Research. 54(4): 452-455.

Afif, M. F. N., Samsudewa, D., & Muktiani, A. (2023). Correlation Between Body Condition Score with Reproductive Disorders, Reproductive Performance and Estrus Performance in Beef Cattle. Animal Production. 25(3): 209-216.

Alhussein MN, and Dang. 2018. Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. Journal Veterinary World. 11(1): 562-577.

Amrutkar SA, SP Pawar, SS Thakur, NJ Kewalramani and MS Mahesh. 2015. Dietary supplementation of rumen-protected methionine, lysine and choline improves lactation performance and blood metabolic profile of Karan-Fries cows. Agricultural research. 4: 396-404.

Ardalan M, K Rezayazdi and M Dehghan-Banadaky. 2009. Investigation on the effect of supplementing rumen-protected forms of methionine and choline on health situation and reproductive performance of Holstein dairy cows. Pakistan Journal of Biological Sciences: PJBS. 12(1): 69-73.

Arifin Z. 2011. Beberapa unsur mineral esensial mikro dalam sistem biologi danmetode analisisnya. Jurnal Litbang Pertanian. 27 (3) : 99-105.

Atabany A, BP Purwanto, TToharmat and A Anggraeni. 2011. Hubungan masa kosong dengan produktivitas pada sapi perah Friesian Holstein di Baturraden, Indonesia. Media peternakan. 34(2): 77-77.

Baumgard LH, LJ Odens, JK Kay, RP Rhoads, MJVanBaale and RJ Collier. 2006. Does negative energy balance (NEBAL) limit milk synthesis in early lactation. In Proc. Southwest Nutr. Conf (pp. 181-187).

Budiawan A, MN Ihsan and S Wahjuningsih. 2015. Hubungan body condition score terhadap service per conception dan calving interval sapi potong Peranakan Ongole di Kecamatan Babat Kabupaten Lamongan. TERNAK TROPIKA Journal of Tropical Animal Production, 16(1): 34-40.

Çetin İ, II Turkmen, KARA Cagdas, AOrman and SEN Erkan. 2018. Improved lactational performance in dairy cows supplemented with methionine or rumen-protected choline during the transition period. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 24(2): 289-293.

Chavda MR, HH Savsani, MR Gadariya, KB Vala, AJ Dhami and VK Karangiya. 2022. Effect of peripartum supplementation of rumen protected choline and rumen protected fat on energy status, Insulin, IGF-1 and postpartum fertility in Gir cows. Indian Journal of Veterinary Sciences & Biotechnology. 18(1): 34-38.

Davidson S, BA Hopkins, J Odle, C Brownie, V Fellner, and LW Whitlow. 2008. Supplementing limited methionine diets with rumen-protected methionine, betaine, and choline in early lactation Holstein cows. Journal of Dairy Science. 91(4): 1552-1559.

Elek P, JR Newbold, T Gaal, L Wagner and F Husveth. 2008. Effects of rumen-protected choline supplementation on milk production and choline supply of periparturient dairy cows. Animal. 2(11): 1595-1601.

Elsaadawy SA, Z Wu, H Wang, MD Hanigan and D Bu. 2022. Supplementing ruminally protected lysine, methionine, or combination improved milk production in transition dairy cows. Frontiers in Veterinary Science. 9: 780637.

Grummer RR. 2008. Nutritional and management strategies for the prevention of fatty liver in dairy cattle. The Veterinary Journal. 176(1): 10-20.

Guretzky NJ, DB Carlson, JE Garrett and JK Drackley. 2006. Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. Journal of dairy science. 89(1): 188-200.

Hartwell JR, MJ Cecava, and SS Donkin. 2000. Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. Journal of dairy science. 83(12): 2907-2917.

Hendrawan VF, A Firmawati, D Wulansari, Y Oktanela, and GC Agustina. 2019. Pemberian vitamin sebagai penanganan gangguan reproduksi sapi kelompok ternak Desa Babakan, Kecamatan

Karangploso, Kabupaten Malang. Jurnal Nutrisi Ternak Tropis. 2(1): 63-69.

Izquierdo CA, VMX Campos, CGR Lang, JAS Oaxaca, SCSuares, CAC Jimenez, MSCJimenez, SDP Betancurt and JEG Liera. 2008. Effect of the offsprings sex on open days in dairy cattle. Journl of Animal and Veterinary Advances. 7: 1329-1331.

Jaenudin D, AA Amin, MA Setiadi, H Sumarno and S Rahayu, S. 2018. Hubungan temperatur, kelembaban, dan manajemen pemeliharaan terhadap efisiensi reproduksi sapi perah di Kabupaten Bogor. Acta Veterinaria Indonesiabna. 6(1): 16-23.

Lanyasunya TP, HH Musa, ZP Yang, DM Mekki and EA Mukisira. 2005. Effects of poor nutrition on reproduction of dairy stock on smallholder farms in the tropics. Pakistan Journal of Nutrition. 4(2): 117-122.

Lunagariya PM, SV Shah and YG Patel. 2023. Lactation Performance of Dairy Cows on Feeding Rumen Protected Choline. The Indian Journal of Animal Sciences. 93(9): 923–927.

McArt JAA, DV Nydam and MW Overton. 2015. Hyperketonemia in early lactation dairy cattle: A deterministic estimate of component and total cost per case. Journal of dairy science. 98(3): 2043-2054.

Mečionytė I, G Palubinskas, L Anskienė, R Japertienė, R Juodžentytė and V Žilaitis. 2022. The Effect of Supplementation of Rumen-Protected Choline on Reproductive and Productive Performances of Dairy Cows. Animals. 12(14): 1807.

Morrison EI, H Reinhardt, H Leclerc, TJ DeVries and SJ LeBlanc. 2018. Effect of rumen-protected B vitamins and choline supplementation on health, production, and reproduction in transition dairy cows. Journal of dairy science. 101(10): 9016-9027.

Nuryadi N and S Wahjuningsih. 2012. Penampilan reproduksi sapi peranakan ongole dan peranakan limousin di Kabupaten Malang. TERNAK TROPIKA Journal of Tropical Animal Production. 12(1): 76-81.

Piepenbrink MS and TR Overton. 2003. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. Journal Dairy Science. 86: 1722–1733

Pirestani A and M Aghakhani. 2018. The effects of rumen-protected choline and l-carnitine supplementation in the transition period on reproduction, production, and some metabolic diseases of dairy cattle. Journal of Applied Animal Research. 46(1): 435-440.

Potts SB, CM Scholte, KM Moyes and RA Erdman. 2020. Production responses to rumen-protected choline and methionine supplemented during the periparturient period differ for primi-and multiparous cows. Journal of dairy science. 103(7): 6070-6086.

Prihatminingsih GE, P Agung and WH Dian. 2015. Hubungan antara konsumsi protein dengan produksi, protein dan laktosa susu kambing Peranakan Etawa. Jurnal Ilmu-ilmu Peternakan. 25 (2) : 20-27.

Rahmani M, M Dehghan-Banadaky and R Kamalyan. 2014. Effects of Feeding Rumen Protected Choline and Vitamin E on Milk Yield, Milk Composition, Dry Matter Intake, BodyCondition Score and Body Weight in Early Lactating Dairy Cows. Iranian Journal of Applied Animal Science. 4(4): 693-698.

Sun F, Y Cao, C Cai, S Li, C Yu and J Yao. 2016. Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. PloS one. 11(8): e0160659.

Sutiyono DS and A Suryawijaya. 2017. Identifikasi gangguan reproduksi sapi betina di peternakan rakyat. Jurnal Veteriner. 18(4): 580-588.

Toledo MZ, GM Baez, A Garcia-Guerra, NE Lobos, JN Guenther, E Trevisoland MC Wiltbank. 2017. Effect of feeding rumen-protected methionine on productive and reproductive performance of dairy cows. PLoS One. 12(12): e0189117.

Tyler HD and ME Ensminger. 2006. Dairy cattle science (No. 636.214 TYLd). Upper Saddle River, NJ, USA:: Pearson prentice hall.

Utomo B and DP Miranti. 2010. Tampilan produksi susu sapi perah yang mendapat perbaikan manajemen pemeliharaan. Caraka Tani. 25 (1): 21 – 25.

Wahyudi L, T Susilawati and S Wahyujingsih. 2013. Tampilan reproduksi sapi perah pada berbagai paritas di Desa Kemiri Kecamatan Jabung Kabupaten Malang. TERNAK TROPIKA Journal of Tropical Animal Production. 14(2): 13-22.

Xu G, JA Ye, J Liu and Y Yu. 2006. Effect of rumen-protected choline addition on milk performance and blood metabolic parameters in transition dairy cows. Asian-Australasian Journal of Animal Sciences. 19(3): 390-395.

Zahra LC, TF Duffield, KE Leslie, TR Overton, D Putnam and SJ LeBlanc. 2006. Effects of rumen-protected choline and monensin on milk production and metabolism of periparturient dairy cows. Journal of dairy science. 89(12): 4808-4818.

Zhou Z, M Vailati-Riboni, E Trevisi, JK Drackley, DN Luchini and JJ Loor. 2016. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. Journal of dairy science. 99(11): 8716-8732.

Zom RLG, J Van Baal, RMA Goselink, JA Bakker, MJ De Veth and AM Van Vuuren. 2011. Effect of rumen-protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy cattle. Journal of dairy science. 94(8): 4016-4027.