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Meta analysis: The impact of utilizing rumen undegradable protein in feed on reproductive and production performance of dairy cow

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ABSTRACT

The purpose of this research was to assess the reproductive and productive parameters of dairy cows with different levels of RUP. This study employed a methodology that involved scientific journal articles focusing on S/C, calving interval, conception rate, PPE, milk yield, milk protein, milk fat, milk lactose, and DMI. The findings indicated no significant differences in milk yield, composition, and reproductive metrics between cows administered RUP and those that were not (p>0.05). According to the NRC (2001), RUP levels constitute 6-10% of the total protein requirement for dairy cows. This study recommends that future research should ensure dairy cows receiving RUP are given the optimal dosage suited to their needs for more precise dosing.

Introduction

Dairy cows are one of the commodities that provide animal protein, and their maintenance is primarily aimed at increasing milk production. In Indonesia, fresh milk production accounts for approximately 25% of the national milk demand, with an average milk consumption of approximately 6 liters per capita per year (Karuniawati and Fariyanti, 2013). Therefore, the majority of national milk demand is still met through imported milk, either as raw materials or processed products. This highlights the underlying issues of the low production and reproductive performance of dairy cows. Low milk production typically occurs near the end of lactation period. However, during the postpartum period or the phase between giving birth and peak lactation, suboptimal milk production can also occur, often because of poor quality feed.

Low-quality feed results in nutrient deficiencies, forcing livestock to utilize the nutrient reserves within their bodies. Feed is provided to dairy cows to meet their nutrient requirements, with the primary needs being energy and protein. These nutrients are essential for maintenance, pregnancy, and milk production. Protein intake is particularly critical, as it supports growth, tissue maintenance (including mammary glands), and the synthesis of amino acids necessary for protein formation (Nugraha *et al*., 2024).

The consumed protein affects dry matter intake in dairy cows. Feeds rich in protein are more likely to be consumed in quantities that fulfill nutritional requirements. Additionally, proteins play a crucial role in maintaining the balance of rumen microflora. They serve as a nitrogen source for rumen bacteria and aid in the development of rumen microbes. Consequently, a higher protein content in the feed promotes the growth of rumen bacteria (Thaariq, 2017). Rumen Undegradable Protein (RUP) is especially important as it improves both the quantity and quality of protein provided by feed and rumen microbes, which is essential for cows with high milk production during lactation.

In livestock, protein metabolism involves rumen microorganisms that transform a portion of the ingested protein into microbial proteins (Robo *et al*., 2019). The intake of protein significantly affects microbial activity by converting amino acids and ammonia into microbial proteins (Annison *et al*., 2002). Furthermore, glucogenic amino acids generate glucose, which is crucial for lactose synthesis, and an increase in lactose production results in higher milk yield (Santosa *et al*., 2009). Amino acids derived from RUP synthesis also impact the enzymatic activity involved in milk fat synthesis within the mammary glands.

Previous research has shown varying outcomes regarding the utilization of RUP in dairy cows. Aboozar and Niazi (2013) reported significant differences in milk production (35–38 kg/day) with RUP levels of 8% and 9%. Conversely, Stevens *et al*. (2020) observed no significant differences in milk yield (41–43 kg/d) with RUP levels of 5.6% and 6.3%, respectively. Therefore, a meta-analysis is necessary to determine the optimal RUP dosage for dairy cows to ensure their efficient utilization.

Materials and methods

This meta-analysis was carried out in April 2025. The research materials included accredited international journals sourced from Google Scholar, Scopus, Science Direct, and other pertinent academic databases. By searching for journals using keywords such as Rumen Undegradable Protein (RUP), dry matter intake (DMI), milk yield, milk protein, milk fat, milk lactose, service per conception (S/C), conception rate (CR), calving interval (CI), and days to first estrus postpartum (PPE) from 1996 to 2024, 23 relevant journals were identified for inclusion in the meta-analysis. The tools employed comprised Microsoft Excel for data collection, Review Manager 5.4 for data interpretation, and RStudio 4.4.2 for conducting the meta-analysis. The method used in this study was quantitative meta-analysis. This study followed nine stages of meta-analysis: determining the topic, collecting literature according to the inclusion and exclusion

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criteria, data extraction, heterogeneity test, hypothesis testing with summary effect, moderator variable analysis, publication bias evaluation, and reporting results.

Data Selection based on Inclusion and Exclusion Criteria

Data search using keywords such as dairy cows, RUP, milk yield, milk protein, milk fat, milk lactose, dry matter intake (DMI), service per conception (S/C), conception rate (CR), calving interval (CI), and postpartum estrus (PPE) resulted in 266 articles. Articles were selected based on the inclusion and exclusion criteria. Inclusion criteria were met if they were from international journals published between 1996 and 2025, contained mean and standard deviation (SD) data, included moderator data such as dosage, and resulted in a final total of 23 articles to be used in the meta-analysis study. The collected data were processed using Microsoft Excel. This step involved adding data on authors, publication year, mean data, standard deviation (SD), moderator data and sample size (n). Data on milk production and milk fat parameters were extracted from seven articles, resulting in 21 studies; milk protein parameters were extracted from six articles, resulting in 17 studies; milk lactose parameters were extracted from four articles, resulting in 11 studies; and DMI parameters were extracted from five articles, resulting in 13 studies. Reproductive parameters (S/C) were extracted from 7 articles resulting in 14 studies, conception rate was extracted from 5 articles resulting in 10 studies, CI parameters were extracted from 3 articles resulting in 7 studies, and PPE parameters were extracted from 4 articles resulting in 10 studies. Article selection was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) method with Review Manager 5.4.1, as shown in Figure 1.

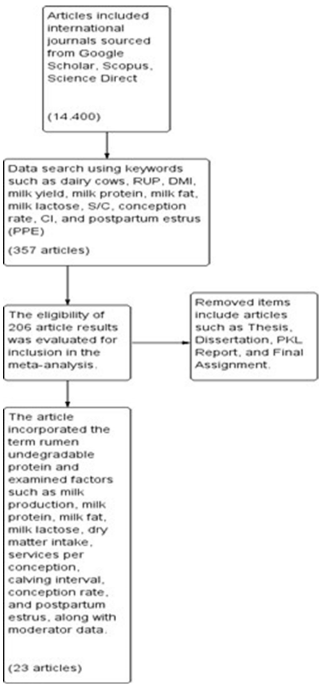

Fig 1. PRISMA diagram

Statistical analysis

Data that were included and excluded underwent meta-analysis using RStudio 4.4.2. Heterogeneity tests are classified under a random effects model if the p-value is significantly different ($p<0.05$), whereas they fall under a fixed-effects model if the p-value is not significantly different ($p>0.05$). The I^2 value, ranging from 0 to 100%, was used to evaluate study heterogeneity, indicating the extent of data variation across studies. The effect size was determined by analyzing the variance between control and experimental groups, using the standardized mean difference to calculate the summary effect. The summary effect is categorized as strong if the value is ≥ 0.5, moderate if it is between 0.5 and 0.3, and weak if it is between 0.3 and 0.1 (Cohen, 1988). Meta-regression was employed for moderator analysis to assess the RUP dosage administration in dairy cows. Publication bias in the meta-analysis was evaluated using funnel plots and Egger's regression test.

Results

Milk yield

In Table 1, there are 21 studies examining milk production parameters. The control group data indicated a milk yield of 39.22 liters per day, whereas the data with RUP administration showed a yield of 39.86 liters per day. The meta-analysis results revealed no significant difference in milk production between the control group and those receiving RUP, as the (p-value>0.05; p=0.628), with study heterogeneity at 32.67%, suggesting homogeneity in the data. Additionally, the meta-regression findings in Table 2 demonstrated that the RUP dosage as a moderator variable had a significant impact ($p<0.05$) on milk production, with a slope of -0.174, indicating that an increase in RUP dosage corresponded to a decrease in milk production, despite its statistical significance.

Table 1. Meta-Analysis Calculation Results for Production Parameters.

Parameters	Number of studies	Control	RUP	Model Estimates	
				ES	p-value
Milk yield (l/day)	21	39.22	39.86	-0.04	0.63
Milk protein (%)	17	3.01	3.01	-0.09	0.60
Milk fat (%)	21	3.39	3.28	0.12	0.29
Milk lactose (%)	11	4.43	4.33	0.18	0.10
DMI (kg/day)	13	22.6	22.75	-0.18	0.05

Milk protein

In Table 1, there were 17 studies examining milk protein parameters. Both the control group and the RUP administration group had a milk protein content of 3.01%. The meta-analysis revealed no significant difference in milk protein content between these two groups, as indicated by a (p-value>0.05; p=0.602). The studies showed heterogeneity (I2 = 68.50%), suggesting that the data were consistent. Additionally, the meta-regression results in Table 2 indicated that the RUP dosage as a moderator variable did not have a significant impact ($p>0.05$), with a slope of -0.007. This suggests that although an increase in RUP dosage was associated with a decrease in milk protein content, this effect was not statistically significant.

Table 2. Meta-Regression Calculation Results for Production Parameters.

Parameters	Number of studies	Model Estimates		
		Slope	SE Slope	p-value
Milk yield	21	-0.17	0.05	0.00
Milk protein	17	-0.01	0.10	0.94
Milk fat	21	0.05	0.07	0.50
Milk lactose	11	-0.02	0.08	0.79
DMI	13	0.03	0.07	0.70

Milk fat

In Table 1, there were 21 studies examining milk fat parameters. The control group data indicated a milk fat content of 3.39%, whereas the group receiving RUP showed a content of 3.28%. The meta-analysis results demonstrated no significant difference in milk fat content between the control and RUP groups, as the (p-value>0.05; p=0.291), with a heterogeneity index of 53.03%, suggesting the data were homogeneous. Additionally, Table 2 illustrates that the meta-regression analysis found the RUP dosage as a moderator variable had no significant impact (p>0.05), with a slope of 0.046, implying that although an increase in RUP dosage corresponded to a rise in milk fat content, this effect was not statistically significant.

Milk lactose

Table 1 includes data from 11 studies examining milk lactose parameters. The control group had a milk lactose content of 4.43%, compared to 4.33% in the group receiving RUP. The meta-analysis showed no significant difference in milk lactose content between the control and RUP groups, as indicated by a (p-value>0.05; p=0.095), with a heterogeneity measure of I2= 3.75%, suggesting the data were consistent across studies. Additionally, Table 2 presents meta-regression findings, which demonstrated that the RUP dosage as a moderator variable did not significantly impact the results (p>0.05). The slope value of -0.021 suggests that an increase in RUP dosage correlates with a decrease in milk lactose content, although this relationship was not statistically significant.

Dry matter intake (DMI)

The total number of studies examining dry matter intake (DMI) parameters in Table 1 is 13. The control group data indicated a DMI of 22.60 kg/day, whereas the data with RUP administration showed a DMI of 22.75 kg/day. The meta-analysis results revealed no significant difference in DMI between the control group and those receiving RUP, as the (p-value>0.05; p=0.052), with study heterogeneity (I2 = 37.64%) suggesting homogeneity in the data. According to the meta-regression results in Table 2, the moderator variable of RUP dosage did not have a significant impact (p>0.05), with a slope value of 0.027, indicating that although higher RUP dosages were associated with increased DMI, this effect was not statistically significant.

Service per conception (S/C)

In Table 3, there were 14 studies examining the service per conception (S/C) parameter. The control group exhibited an S/C value of 2.20, while the group receiving RUP had a value of 2.24. The meta-analysis results showed no significant difference in S/C between the control and RUP groups, as indicated by a (p-value>0.05; p=0.315), with no heterogeneity among the studies (I2 = 0%), suggesting the data were consistent. According to Table 4, the meta-regression analysis found that the RUP dosage as a moderator variable did not have a significant impact (p>0.05), with a slope of 0.006, suggesting that although an increase in RUP dosage corresponded to a higher S/C value, this effect was not statistically significant.

Table 3. Meta-Analysis Calculation Results for Reproductive Parameters.

Parameters	Number of studies	Control	RUP	Model Estimates	
				ES	p-value
S/C	17	2.2	2.24	-0.04	0.32
CI	7	394	393	-0.12	0.13
CR	10	36.16	46.54	-1.67	0.09
PPE	10	51.97	56.61	-0.28	0.36

Calving interval (CI)

Table 3 includes data from seven studies examining the calving interval (CI) parameter. The control group exhibited a CI of 394, while the group receiving RUP had a CI of 393. The meta-analysis revealed no significant difference in CI between the control and RUP groups, with a (p-value>0.05; p=0.130). The studies showed heterogeneity (I2 = 48.92%), suggesting the data were homogeneous. According to the meta-regression results in Table 4, the RUP dosage as a moderator variable did not significantly impact the CI (p>0.05), with a slope of -0.022. This indicates that although an increase in RUP dosage was associated with a decrease in CI, the effect was not statistically significant.

Table 4. Meta-Regression Calculation Results for Reproductive Parameters.

Parameters	Number of studies	Model Estimates		
		Slope	SE Slope	p-value
S/C	17	0.01	0.01	0.50
CI	7	-0.02	0.12	0.85
CR	10	-0.17	0.05	0.00
PPE	10	-0.13	0.13	0.35

Conception rate (CR)

Table 3 presents data from 10 studies examining the conception rate (CR) parameter. The control group exhibited a CR of 36.16, whereas the RUP group had a CR of 46.54. According to the meta-analysis, there was no significant difference in CR between the control and RUP groups, as indicated by a (p-value>0.05; p=0.091). The studies showed considerable heterogeneity (I2 = 99.35%), suggesting variability in the data. Table 4's meta-regression results reveal that the RUP dosage as a moderator variable significantly influenced the CR (p<0.05), with a slope of -0.174, implying that an increase in RUP dosage corresponds to a decrease in CR.

Pregnancy per insemination (PPE)

Table 3 includes data from 10 studies examining pregnancy per insemination (PPE) parameters. The control group exhibited a PPE value of 51.97, while the group receiving RUP showed a PPE of 56.61. The meta-analysis revealed no significant difference in PPE between the control and RUP groups, as indicated by a (p-value>0.05; p=0.356), with a high level of heterogeneity among the studies (I2 = 92.94%), suggesting variability in the data. According to the meta-regression results in Table 4, the RUP dosage as a moderator variable did not have a significant impact (p>0.05), with a slope of -0.125, indicating that an increase in RUP dosage corresponded to a decrease in PPE value, although this relationship was not statistically significant.

Discussion

Rumen non-degradable protein (RUP) is a protein that is not degraded by rumen microbes. RUP, along with microbial proteins, is digested in the abomasum with the help of pepsin to produce polypeptides. These polypeptides are further digested in the small intestine, resulting in amino acids. Amino acids (AA) are then absorbed and used as substrates for the formation of protein hormones. Amino acids are transported through the bloodstream to the hypothalamus, where they trigger the release of gonadotropin-releasing hormone (GnRH). This hormone then travels via the blood to several organs, such as fat cells, the stomach, pancreas, and pituitary gland. In the pancreas, GnRH prompts the production of insulin, which is crucial for managing glucose metabolism. Within fat cells, GnRH encourages the production of leptin, a hormone that helps regulate energy balance and brain signaling related to body fat levels. Furthermore, it stimulates the stomach to release ghrelin, a hormone involved in appetite stimulation and energy regulation.

GnRH also prompts the pituitary gland to secrete insulin-like growth factor-1 (IGF-1) and release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Prolactin aids in the development of the mammary gland and milk production post-birth, while oxytocin induces uterine contractions during childbirth and stimulates milk alveoli during milk ejection. These hormones are vital for the production and reproduction processes in dairy cows. The hormones significant in dairy cow production include ghrelin, leptin, prolactin, and oxytocin, which collectively influence the outcomes of dairy cow production.

There was no significant difference in milk production between dairy cows that received Rumen Undegradable Protein (RUP) and those that did not. This could be attributed to the high RUP content, which elevates amino acid levels in the body. When there is an amino acid surplus, cows convert them into urea, which is subsequently expelled through urine (Getahun *et al*., 2019) . This suggests a mismatch between protein and energy nutrition levels. As a result, the energy that should be allocated for lactose synthesis is instead used for urea production. Since lactose controls milk volume, this impacts the quantity of milk produced (Vidyanto *et al*., 2015). At specific doses, RUP can be effectively utilized by livestock through protein metabolism. However, excessive RUP due to an imbalanced amino acid profile has been found to reduce the efficiency of metabolizable protein use for milk protein synthesis, as the protein's nutritional value is contingent on its essential amino acid profile (Lanzas *et al*., 2008).

Milk protein results in dairy cows given and not given Rumen Undegradable Protein (RUP) do not differ significantly, and this occurs because of an imbalance in the nutrients of the feed. If RUP is provided in high amounts without considering the balance between Rumen Degradable Protein (RDP) and energy, rumen microbes will lack nitrogen for rumen fermentation. This disrupted fermentation results in decreased production of Volatile Fatty Acids (VFA), reducing the availability of energy, especially glucose, for milk protein synthesis. Milk protein synthesis requires specific amino acids such as lysine and methionine. If the provided RUP does not have a balanced amino acid profile that meets the requirements, the amino acids from the RUP do not optimally support an increase in milk protein content. This contrasts with the statement by Aboozar and Niazi (2013), who stated that the increase in milk protein in dairy cows administered RUP at appropriate doses leads to a good availability of the amino acid profile in milk, resulting in an optimal ratio of lysine and methionine to the small intestine. According to Trinacty *et al*. (2009), this optimal availability of lysine and methionine can protect amino acids from microbial degradation, allowing protected amino acids to pass through the abomasum and small intestine, where they are released and absorbed.

The milk fat content in dairy cows given and not given Rumen Undegradable Protein (RUP) does not differ significantly. This is because feed with a high RUP content produces more protein, reducing the amount of protein available to be broken down in the rumen. This leads to proteolytic microbes producing less ammonia, thereby disrupting the activity of the cellulolytic microbes responsible for fiber fermentation. The disruption in the balance of energy and protein leads to a decline in microbial populations, which hampers the fiber fermentation process and subsequently reduces the production of fermentation products, specifically Volatile Fatty Acids (VFA). These VFAs, including propionic, butyric, and acetic acids, are crucial for synthesizing milk fat (Mutamimah *et al*., 2013). This finding aligns with Palmquist and Weiss (1994), who observed that increasing the RUP dosage results in a linear rise in milk fat percentage. Huhtanen and Hristov (2009) suggest that excessive RUP administration might decrease the digestive efficiency in dairy cows because rumen microbes are unable to break down RUP into necessary amino acids, thereby limiting energy availability for milk fat synthesis.

The lack of variation in milk lactose parameters between the control and Rumen Undegradable Protein (RUP) treatments could be attributed to high RUP levels causing nutritional imbalances, which alter microbial populations. This impacts fiber fermentation and the production of Volatile Fatty Acids (VFA) like propionate, a vital component in milk lactose synthesis (Imanto *et al*., 2018). Lower propionate levels can reduce glucose production, thereby decreasing glucose availability for lactose synthesis (Mustopa *et al*., 2023). Inappropriate or excessive RUP dosages may disrupt the protein and energy balance in dairy cows, adversely affecting milk lactose production. Although RUP is not directly involved in lactose synthesis, sufficient amino acid availability can improve protein metabolism efficiency, reducing the cow's need to break down its own body proteins for amino acids. This, in turn, supports fermentation.

The result of dry matter intake (DMI) are consistent with the study by Yekdangi *et al*. (2014), which demonstrated that administering Rumen Undegradable Protein (RUP) affects DMI in dairy cows. According to Zain *et al*. (2023), this effect might be attributed to the increased protein content in the feed, which satisfies the nutritional requirements of rumen microbes, thus boosting DMI in dairy cows. The research outcomes related to the service per conception (S/C) parameter align with those of Wu and Satter (2000), who reported that a diet high in Rumen Undegradable Protein (RUP) does not impact S/C values. This conclusion is corroborated by Wang *et al*. (2008), who observed that RUP administration can affect nitrogen metabolism and enhance the delivery of essential amino acids to the small intestine. These amino acids effectively support energy production and may aid in reducing the Negative Energy Balance (NEB), thereby speeding up the recovery of the estrus cycle.

The meta-regression findings for the calving interval (CI) parameter align with Keady *et al*. (2001), who observed no notable difference between dairy cows receiving Rumen Undegradable Protein (RUP) and those not receiving it. Administering RUP in suitable amounts can aid in maintaining postpartum energy balance, thus hastening the reproductive cycle. This is consistent with McCormick *et al.* (1999), who noted that excessive crude protein (CP) and RUP levels in dairy cows can impact reproductive efficiency, consequently affecting the calving interval. The reduction in conception rate (CR) among dairy cows given RUP is linked to the specified dosage, which supplies essential amino acids for hormonal and reproductive functions. However, administering too much RUP disrupts livestock reproduction. Excessive RUP results in the conversion of surplus amino acids to urea in the liver. As per Kurniawati *et al*. (2021), elevated blood urea levels alter uterine fluid composition, hinder embryo development, and damage endometrial cells, thereby interfering with embryo implantation.

Conclusion

When incorporating RUP into dairy cow feed, it is essential to adjust the dosage to align with the specific needs of the cows, ensuring that RUP is efficiently used in their metabolic processes.

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Conflict of interest

The authors have no conflict of interest to declare.

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