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EXPLORATIVE STUDY OF BLOOD UREA NITROGEN (BUN), SERUM ESTROGEN, AND CONCEPTION RATE (CR) IN SMALL-SCALE DAIRY FARMS BASED ON QUANTITY OF FEED CONCENTRATE CONSUMED BY THE CATTLE

Sri Mulyati[1], Fachruddin Aziz[2], Aswin R. Khairullah[3], Imam Mustofa[1*], Budi Utomo[1], Pudji Srianto[1], Tri W. Suprayogi[1], Suzanita Utama[1], Wurlina Wurlina[1], Arif N. M. Ansori[4,5,6], Adeyinka O. Akintunde[7], Chairdin D. Nugraha[8], Riza Z. Ahmad[3], Lili Anggraini[8], Latifah Latifah[8]

[1] Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia
[2] Master Program of Reproductive Biology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia
[3] Research Center for Veterinary Science, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia
[4] Postgraduate School, Universitas Airlangga, Surabaya, East Java, Indonesia
[5] Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, Uttarakhand, India
[6] Medical Biotechnology Research Group, Virtual Research Center for Bioinformatics and Biotechnology, Surabaya, East Java, Indonesia
[7] Department of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Nigeria
[8] Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia

Keywords: *services per conception (S/C), milk yield, BUN, estrogen, conception rate*

Abstract

The purpose of this study was to measure the level of BUN in dairy cows and its influence on services per conception (S/C) rate, milk output, and the forage to concentrate (F/C) ratio. Oestrogen concentrations were also assessed based on BUN level and pregnancy rate. Three blood samples were taken for the measurement of estrogen on the day of AI (D0), seven days later (D+7), and twenty-two days later (D+22). From the entire herd, a batch of eighteen dairy cows in total were chosen at random and split to the groups based on S/C, milk output, F/C ratio, BUN, and pregnancy rate. The means of BUN based on F/C ratio showed significant difference (p<0.05) of the results. Each group's estrogen concentration on D0 and D+7 did not significantly differ (p>0.05) in regards to BUN and pregnancy rate, while the group of non-pregnant cows with high BUN featured lower value. However, in D+22, the group of non-pregnant cows with high BUN showed a lower estrogen concentration than the group of pregnant cows with low BUN (p<0.05), while the group of pregnant cows with low BUN had a higher BUN concentration than the group of non-pregnant cows with low BUN (p>0.05). These results indicated that while feed could alter BUN concentrations, non-feeding factors should also be taken into account. S/C and milk yield were found to provide no effect on BUN concentrations. According to these findings, a higher BUN concentration decreased the CR value, and concentrations of BUN ≥18 mg/dL led to lower levels of estrogen.

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Introduction

The success of a dairy farm is determined by feed management factors. Feed should contain nutrients necessary for the cow's body, in sufficient quantity and adequate quality for survival and reproduction. Nutrients needed by cows include carbohydrates, fat, protein, vitamins, water, minerals and inorganic elements [1]. Balanced and sufficient amount of feed according to the animal's needs will ensure optimal productivity and reproductive efficiency [2]. Since protein is the essential element of food primarily required to boost milk production, lactating dairy cows' milk yield can often be raised by increasing the share of protein in their diet [3]. To boost milk yield, dairy farmers as a rule increase the amount of feed given; nevertheless, it is recognized that increasing the amount of feed protein can negatively impact the reproductive functions [4].

Urea is the final byproduct of ruminant protein metabolism that enters the blood circulation [5]. High-yielding dairy cows show decreased fertility, longer days open (DO) and calving intervals (CI), increased service per conceive (S/C) and decreased conceive rate (CR) [6]. Increasing protein concentration in the ration results in increased urea nitrogen concentration in the body. The liver's process of detoxifying ammonia produces urea, a metabolite of protein included into the ration [7]. The circulatory system distributes urea, which passively diffuses across bodily fluids and contains milk urea nitrogen (MUN) and blood urea nitrogen (BUN) [8]. MUN concentration is highly correlated with BUN concentration. As a result, BUN measurement can provide farmers with crucial information regarding the health and nutritional state of the cows [9].

Ineffective intake of nitrogen for development and milk production is indicated by high BUN values [10]. Increased urea nitrogen concentrations have been shown to provide detrimental effects on dairy cow's fertility, including altered uterine fluid pH, impaired ovarian function, mineral imbalance in the uterus, lower rates of conception, and hormonal imbalance leading to reproductive disorders [11]. In addition to impaired immunity, lactating cows' secretion of K, Mg, and P, and causing hormonal imbalances, including estrogen level deviation, high blood urea prevents the removal of uterine contaminations [12]. High levels of urea nitrogen in dairy cows have detrimental effects on reproduction function because they prevent fertilization and follicle growth, decrease the ability of progesterone to attach to the ovarian receptor, and decrease the binding of luteinizing hormone to the ovarian receptor [13].

A number of risk factors, including managerial, environmental, metabolic, and nutritional problems, interact with each other to negatively impact fertility and milk yield. Reproductive function failure or infertility is a complex condition. The amount of protein in the concentrate, the resulting BUN concentration, and its potential detrimental effects on various reproductive processes are a few examples of such factors [14]. The aim of this study was to find the correlation between the level of blood urea nitrogen (BUN), level of serum estrogen and conception rate (CR) with fertility and milk yield criteria in dairy cattle.

Objects and methods

Data and sample collection

The owner of a small-scale dairy farms in Wagir, Malang, East Java, Indonesia has a total population of ± 1.000 lactating dairy cows. Among them one hundred cows were selected randomly based on the dairy cow type [Holstein-Friesian (HF)], milk yield, age, parity, health condition and measured values of body condition score BCS (1–9 points). Fifty cows were further selected based on their reproductive efficiency data [services per conception (S/C), calving interval (CI), days open (DO)] which is supported by the relevant records on cows, and the quantity of feed (forage and concentrate) consumed by them. These 50 cows were then grouped into three groups, based on S/C and milk yield criteria, (S/C 1–2, milk yield < 17 L/day), (S/C 3–4, milk yield 17–21 L/day) and (S/C ≥ 5, milk yield > 21 L/day). Milk yield ranges were calculated from< (means – SD); between (means – SD) and (means + SD), and ≥ (means + SD), while S/C were divided accordingly. Among the 50 cows, 18 cows complied the three groups criteria (6 cows in each group)

Blood samples were taken through the coccygeal vein (5 mL from each cow). Blood sample for estrogen measurement was taken three times, which happened on the day of AI (*D*0), seven days after AI (*D* + 7), 22 days after AI (*D* + 22), and blood sampling for BUN measurement was taken only once. Two hours after collection, blood samples were centrifuged at 2.000 rpm for 10 minutes. Serum was separated and stored (–20 °C). Pregnancy was tested 3 months after AI, through rectal palpation. After all data were obtained, regrouping was done to determine differences in the concentration of BUN and CR based on the F/C ratio, and the concentration of BUN (<18 mg/dL; ≥ 18 mg/dL) and rate of pregnancy on estrogen concentrations parameter.

Measurement of BUN and estrogen

BUN was measured using Berthelot method by Balai Besar Laboratorium Kesehatan Surabaya, while estrogen was measured by the lab instrument ELISA (DRG instrument GmbH, Germany) at the Laboratory of Endocrinology, Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga Surabaya.

Statistical analysis

Analysis was performed to identify the homogenity based on age, parity, milk yield and BCS parameters. Similarly, the homogenity of samples data of 18 cows which was further explored from among 50 cows was studied based on reproductive efficiency and composition of feed consumed. The SPSS23.0 software was used to conduct statistical analyses using One Way ANOVA and the Independent-Samples T Test ($p < 0.05$).

Results and discussion

Data collection of 100 dairy cows from a herd of approximately 1,000 lactating dairy cows in Wagir, Malang, East Java, Indonesia, showed range of ages between 3–8 years, parity 2–5, BCS 4–7 and milk yield 8–29 L/day. Then, 50 out of the 100 cows were surveyed and found to have S/C, CI and DO respectively 1–8 times, 338–697 days and 37–98 days range with a mean of 4.12 ± 0.31 times, 454.31 ± 12.9 days and 66.12 ± 2.53 days, and the range of age, parity, BCS and milk yield were respectively 3–7 years, 2–5.4–7 and 10–29 L/day, with a mean of 3.94 ± 0.36 years, 2.22 ± 0.3, 5.11 ± 0.28 and 19.17 L/day respectively. Range of forage and concentrate quantity was 20–70 kg/day and 5–16 kg/day respectively, with a mean of 35.1 ± 1.19 kg/day and 10.26 ± 0.39 kg/day. Then 18 dairy cows were selected from among 50 cows and sorted by groups with means of milk yield, S/C, quantity of forage and concentrate equal to 19.17 ± 2.17 L/day, 3.89 ± 0.83, 34.44 ± 0.66 kg/day and 10.67 ± 1.33 kg/day respectively. Based on the results, it can be said that the number of cows per group can represent the number of dairy cow population at the study site.

S/C, milk yield and BUN

The comparison between each group showed that the mean of S/C and milk yield of dairy cows group with S/C ≥ 5 featured the highest milk yield > 21 L/day. Statistically the mean of S/C and milk yield in each group was significantly different ($p < 0.05$). The group of cows with high milk yield along with high S/C under consideration in this study had the lowest BUN concentration since there was a negative correlation between milk production and urea nitrogen content [15]. However, it was not statistically significant ($p > 0.05$) (Table 1). The concentration of urea nitrogen was not directly related to milk production and was not much affected by lactation period, but it was related to the balance of protein being fed [16,17]. Urea nitrogen concentrations can be utilized as a biomarker of how well lactating cows use nitrogen for milk production because urea nitrogen concentrations are regulated by the amount and concentration of consumed dietary crude protein [18].

Table 1. Milk yield, S/C, BUN (mg/dL), DO, S/C, CI and CR based on quantity of feed concentrate consumed by a cow

	Concentrate < 10.63 kg/d	Concentrate ≥10.63 kg/d	Grand Mean
Concentrate (kg/d)	9.50 ± 1.41^a	11.50 ± 1.31^b	10.63 ± 0.69
Milk yield (L/d)	13.50 ± 0.62^a	25.17 ± 1.19^b	19.00 ± 1.23
BUN (mg/dL)	15.61 ± 1.43^a	15.67 ± 1.29^a	15.64 ± 1.62
% BUN ≥ 18 mg/dL	44.44 % (4/9)^a	22.22 % (2/9)^a	33.33 % (6/18)
DO	61.33 ±5.87^a	64.67 ± 4.86^a	63.95 ±3.74
S/C	1.67 ± 0.21^a	6.33 ± 0.21^b	3.74 ± 0.49
CI	407.33 ± 21.32^a	444.33 ± 34.60^b	422.05 ± 20.04
CR	33.33 % (2/6)^a	33.33 % (2/6)^a	33.33 % (6/18)

Note: Different superscripts on the same row show significant differences ($p < 0.05$).

A high amount of protein feed (rumen degradable and rumen undegradable protein) with a crude protein content of > 19 % [19,20] results in a higher concentration of urea nitrogen (in urine, blood and milk) and a decrease in the efficiency of *N* utilization, which increases the excretion of nitrogen (N) [21–23]. Protein feed can be obtained from the feeding of concentrates. The highest average of F/C ratio was found in the group with low milk yield (< 17 L/day) ($p < 0.05$). This was because the quantity of concentrate is much smaller than the quantity of forage, so the protein nutrient is not enough to increase milk yield. The ratio of forages to concentrates that can increased the milk yield and raise the concentration of milk protein among the dairy cows in early lactation period was 60:40 [24].While based on the mean of concentrate feeding, the highest milk yield was recorded in the group of cows with milk yield > 21 L/day and the lowest one was recorded in the group of cows with milk yield < 17 L/day ($p < 0.05$). It indicated that concentrate supplementation is able to increase milk production [25]. Thus, the lactating cows in this group may have a balance of feed management and efficient utilization of *N* for milk production, without increasing the concentration of urea nitrogen, because a balanced feeding for lactating cows was 14–16 mg/dL in average of BUN concentration [26].

S/C can be affected by the high concentration of BUN [27], but no significant difference ($p > 0.05$) between S/C and BUN concentrations was found in this study. Yoon et al. [28] also reported that there was no effect of nitrogen urea concentration on frequency of successful artificial insemination. The high ratio of S/C (repeated attempts of conception) was not only influenced by BUN but also by fertilization failure and embryonic mortality caused by many factors, among others — the flaws of artificial insemination, environmental issues, ovulatory failure, poor genetics and uterine infection. Therefore, the conception rates in each milk yield and S/C based group also showed no significant difference ($p > 0.05$) [29]. Furthermore, regrouping was performed based on F/C ratio and BUN concentrations, each group with greater and less parameters than the grand mean of CR.

BUN, conception rate and F/C ratio

The mean of forage to concentrate (F/C) ratio and BUN (from grand mean) in this research were equal to 3.52 and 15.64 mg/dL. Then the cows were grouped into: < 3.52; ≥ 15.64 mg/dL, < 3.52; < 15.64 mg/dL, ≥ 3.52; ≥ 15.64 mg/dL and ≥ 3.52; < 15,64 mg/dL, with significant difference ($p > 0.05$) in mean result of BUN concentration. The quantities of forage and concentrates in the F/C ratio ≥ 3.52 group was lower than that of the F/C ratio < 3.52 group ($p < 0.05$), which was linear correlation to the share nutritional protein in each group.

Protein consumption, the effectiveness of *N* use for milk production, and energy balance can all affect a dairy cow's urea nitrogen content [30,31]. The absence of BUN that was ≥ 15.64 mg/dL in F/C ratio ≥ 3.52 in a group indicated that the amount of protein feed given was not high, thus unable to increase the concentration of BUN, whereas in cows that obtained F/C ratio ≥ 3.52 with BUN < 15.64 mg/dL (Table 2). BUN was averagely < 12 mg/dL, which indicated protein deficiency and it was suggested to give additional feed [8]. In cows given F/C ratio < 3.52 BUN concentration were ≥ 15,64 mg/dL. It was caused by the higher concentrate feed F/C ratio < 3.52 than F/C ratio ≥ 3.52 ($p < 0.05$). Adding the concentrate feed may increase the concentration of urea nitrogen [32]. Cows given F/C ratio < 3.52 with BUN < 15.64 mg/dL showed that dairy cows are able to efficiently utilize *N* from feed for productivity, whereas cows given ratio of F/C < 3.52 with BUN ≥ 15.64 mg/dL had a high concentration value of ≥ 18 mg/dL, which showed that there was a decrease in efficiency of utilization *N*, thus increased the excretion of *N* [18,22].

Table 2. Blood urea nitrogen (BUN) (mg/dL) and conception rate (CR) based on quantity of feed concentrate consumed and BUN (mg/dL) were more or less than the mean of whole sample

	Concentrate ≥10.63 kg/d		Concentrate < 10.63 kg/d
	BUN≥15.64	BUN < 15.64	BUN < 15.64
Concentrate (kg/d)	11.43± 0.95^{a}	11.63± 0.88^{a}	6.33 ± 0.88^{b}
BUN(mg/dL)	19.93 ± 0.80^{a}	13.38 ± 0.53^{b}	11.67 ± 1.27^{b}
CR (%)	14 % (1/7)	50 % (4/8)	33.3 % (1/3)

Note: Different superscriptson the same row show significant differences ($p < 0.05$).

In addition to utilizing protein from feed, ruminants can also synthesize proteins on their own with the help of microbes available in rumen. Ruminants can also utilize sources of nitrogen that are not derived from proteins (named non protein nitrogen, NPN), for the synthesis of their body proteins, in which microbial proteins have very high biological values. Thus, the amino acid supply of the body comes from feed proteins and rumen microbes. Although some proteins are resistant to rumen degradation, feed proteins and NPN that ruminants consume partially break down in the rumen, i. e. amino acids break into ammonia and branched chain fatty acids, which then provide amino acids and peptides that cattle can absorb in their intestines and use to boost its productivity [33]. Ammonia is necessary for rumen microorganisms to flourish. The rumen contains an excess of *N-NH3* if amino acids and peptides that have not been utilized for milk production are absorbed. If not utilized for microbial protein synthesis, the excess of *N-NH3* in the rumen is absorbed through the rumen wall, transformed into urea in the liver, and partially excreted in the urine [34]. As a result, cows may not always use high protein diets to their full potential; this relies on how well they process nitrogen to produce milk. If not used nitrogen raises the urea nitrogen concentration [31].

Group of cows which was given F/C ratio < 3.52 with BUN ≥ 15.64 mg/dL showed the lowest CR value. This indicated that the increase of feeding quantity that could increase BUN, in accordance with previous study where BUN concentration > 16 mg/dL resulted to the lower pregnancy rate [35], and BUN ≥ 18 mg/dL could lead to decreased fertility [9]. The group of cow given F/C ratio ≥ 3.52 with BUN < 15.64 mg/dL also had low CR values, which indicated that feeding with low quantity of forage and concentrate could also reduce CR and BUN concentrations.

BUN, estrogen and pregnancy

Pregnancy detection of 18 cows showed that 6 cows were pregnant and the other 12 cows were not pregnant. Previously reported that the decrease of conception rates in dairy cow can be influenced by the high concentration of BUN that reach ≥ 18 mg/dL [36] while half of the 12 non-pregnant cows had BUN concentrations of ≥ 18 mg/dL in accordance to the results of this study. Pregnant cows in this study had a mean concentration of BUN < 18 mg/dL, reflected they could achieve maximum fertility if the concentration of urea nitrogen ranged within 12–16 mg/dL [37]. The lack of pregnancy of cows with BUN < 18 mg/dL may be caused by other factors that contributed the repeated attempts of conception. Cows were divided into three groups according to their BUN and pregnancy status, they were grouped to low BUN and pregnant, high BUN and non-pregnant and low BUN and non-pregnant with mean concentrations of 13.92, 20.42, and 12.58 mg/dL of BUN respectively. Statistically the group of low BUN and pregnant and the group of low BUN and non-pregnant group showed no significant difference between each other ($p > 0.05$), but significantly different from the group of high BUN, non-pregnant cows ($p < 0.05$).

High BUN concentrations in dairy cows may be due to intense feeding with crude protein (CP). There are several reasons why too much dietary CP reduced reproductive performance [9,38]. Excess dietary CP has been linked to poor energy status because it can raise energy requirements, which can range from 13.3 kcal of digested energy per gram of excess *N*. Due to delayed ovulation and decreased plasma progesterone levels, poor energy status may decrease fertility. Sperm, oocytes, and embryos may be toxically affected by high BUN concentrations. It has also been noted that high BUN levels reduce the formation of prostaglandin (PGF2α), luteal phase P, Mg, and K concentrations, and pH of uterine fluid. Additionally, a high BUN may lessen the binding of leutinizing hormone (LH) to ovarian receptors. Reduced LH binding would result in lower levels of progesterone in the blood, which would lower fertility [39,40].

Estrogen concentration measured on D(0) showed that its value in the group of low BUN and non-pregnant cows < the group of high BUN and non-pregnant cows < the group of low BUN and pregnant cows; and on D(+7) it changed the following way: the group of high BUN and non-pregnant cows < the group of low BUN and pregnant cows < the group of low BUN and non-pregnant cows, but each group showed no significant difference in estrogen concentration ($p > 0.05$), whereas on D(+22), estrogen concentrations in the group of low BUN and pregnant cows was lower than the group of low BUN and non-pregnant cows and showed significant difference ($p < 0.05$), but a mean of estrogen concentration on D(+22) in group of low BUN and pregnant cows and the group of high BUN and non-pregnant cows were not significantly different ($p > 0.05$) (Figure 1).

Cows with BUN concentrations of ≥ 18 mg/dL showed false positive pregnancies because estrogen concentrations in blood were not significantly different from the pregnant cows with BUN concentrations < 18 mg/dL, they also had a lower estrogen concentrations than pregnant and not pregnant cows with BUN concentrations < 18 mg/dL. Silva et al. [41] reported that there was a negative correlation between the concentrations of urea nitrogen and estrogen, and higher protein intake (undegradable protein) is able to cause low estrogen concentrations. High feed intake can increase excessive blood flow to the gastrointestinal tract

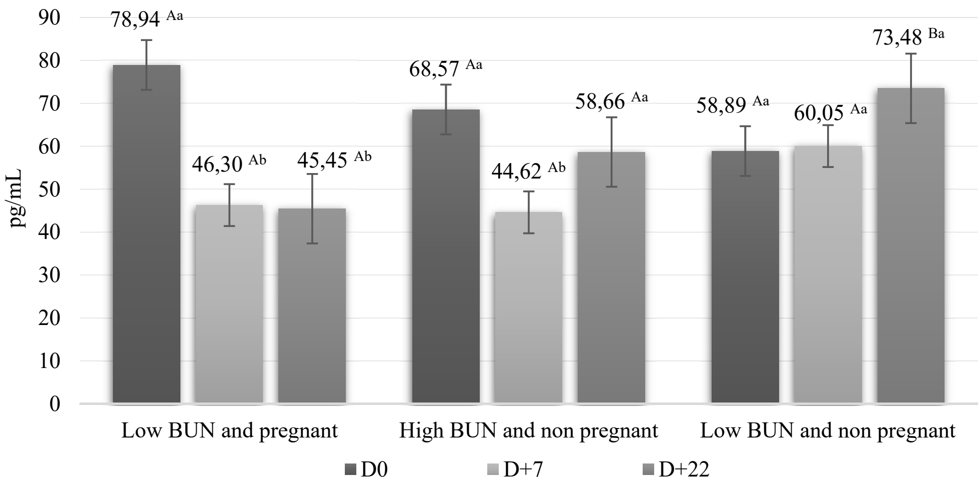

Figure 1. Serum estrogen levels (pg/mL) at day-0, day-7 and day-22 (day-0 = estrus) grouped by BUN and pregnancy status; Different superscripts ($^{A,\,B}$) show significant differences ($p < 0.05$) between groups; Different superscripts ($^{a,\,b}$) show significant differences ($p < 0.05$) between day of sampling

and to the liver. The liver has a major function for the metabolism of progesterone and estradiol 17β, increased liver blood flow causes cessation of hormone metabolism in the liver, thus lowering the concentrations of progesterone and estrogen in the blood [42]. High concentrations of urea nitrogen may also affect hormonal secretion in the ovaries, namely decreased the concentrations of luteinizing hormone (LH) that binds to ovarian receptors, insulin and insulin-like growth factor-1 (IGF-1), which all three hormones play a role in the process of steroidogenesis [43]. The IGF-1 and insulin stimulate cells proliferation and differentiation, and act synergistically with FSH in steroidogenesis by increasing the activity of P450 aromatase, and increase the secretion of estradiol. In addition, LH is also a stimulator of aromatase activity in granulosa cells, along with its being the physiological factor in the follicular ovarian of estradiol production regulator (E2) in cattle [44]. Reduced reproductive function could result from the product of *N* metabolism changing the hypophyseal pituitary-ovarian axis's activity [28].

Conclusion

The results of this study confirm that the occurrence of pregnancy can be affected by BUN concentration as the factor altering estrogen concentration. An increased concentrations of BUN ≥18 mg/dL showed false positive pregnancy, based on estrogen concentration, and caused lowered conception rates.

REFERENCES

1. Erickson, P. S., Kalscheur, K. F. (2020). Nutrition and feeding of dairy cattle. Chapter in a book: Animal Agriculture. Sustainability, Challenges and Innovations. Academic Press, 2020. https://doi.org/10.1016/B978-0-12-817052-6.00009-4
2. Maltz, E., Barbosa, L. F., Bueno, P., Scagion, L., Kaniyamattam, K., Greco, L.F. et al. (2013). Effect of feeding according to energy balance on performance, nutrient excretion, and feeding behavior of early lactation dairy cows. *Journal of Dairy Science*, 96(8), 5249–5266. https://doi.org/10.3168/jds.2013-6549
3. Tyasi, T. L., Gxasheka, M., Tlabela, C. P. (2015). Assessing the effect of nutrition on milk composition of dairy cows: A review. *International Journal of Current Science*, 5(2), 56–63.
4. Martens, H. (2023). Invited Review: Increasing milk yield and negative energy balance: A gordian knot for dairy cows? *Animals*, 13(19), Article 3097. https://doi.org/10.3390/ani13193097
5. Weiner, I. D., Mitch, W. E., Sands, J. M. (2015). Urea and ammonia metabolism and the control of renal nitrogen excretion. *Clinical Journal of the American Society of Nephrology*, 10(8), 1444–1458. https://doi.org/10.2215/CJN.10311013
6. Carty, C. I., McAloon, C. G., O'Grady, L., Ryan, E. G., Mulligan, F.J. (2020). Relative effect of milk constituents on fertility performance of milk-recorded, spring-calving dairy cows in Ireland. *Journal of Dairy Science*, 103(1), 940–953. https://doi.org/10.3168/jds.2018-15490
7. Cholico, G.N., Fling, R. R., Sink, W. J., Nault, R., Zacharewski, T. (2024). Inhibition of the urea cycle by the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin increases serum ammonia levels in mice. *Journal of Biological Chemistry*, 300(1), Article 105500. https://doi.org/10.1016/j.jbc.2023.105500
8. Arunvipas, P., VanLeeuwen, J. A., Dohoo, I. R., Keefe, G. P., Burton, S. A., Lissemore, K. D. (2008). Relationships among milk urea-nitrogen, dietary parameters, and fecal nitrogen in commercial dairy herds. *Canadian Journal of Veterinary Research*, 72(5), 449–453.
9. Rajala-Schultz, P. J., Saville, W. J. A., Frazer, G. S., Wittum, T. E. (2001). Association between milk urea nitrogen and fertility in Ohio dairy cows. *Journal of Dairy Science*, 84(2), 482–489. https://doi.org/10.3168/jds.s0022-0302(01)74498-0
10. Roggero, P., Giannì, M. L., Morlacchi, L., Piemontese, P., Liotto, N., Taroni, F. et al. (2010). Blood urea nitrogen concentrations in low-birth-weight preterm infants during parenteral and enteral nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, 51(2), 213–215. https://doi.org/10.1097/mpg.0b013e3181cd270f
11. Cheng, Z., Oguejiofor, C. F., Swangchan-Uthai, T., Carr, S., Wathes, D. C. (2015). Relationships between circulating urea concentrations and endometrial function in postpartum dairy cows. *Animals*, 5(3), 748–773. https://doi.org/10.3390/ani5030382
12. Sheldon, I.M., Cronin, J., Goetze, L., Donofrio, G., Schuberth, H.-J. (2009). Defining postpartum uterine disease and the mechanisms of infection and immunity in the female repro-

ductive tract in cattle. *Biology of Reproduction*, 81(6), 1025–1032. https://doi.org/10.1095/biolreprod.109.077370

13. Sammad, A., Khan, M. Z., Abbas, Z., Hu, L., Ullah, Q., Wang, Y. et al. (2022). Major nutritional metabolic alterations influencing the reproductive system of postpartum dairy cows. *Metabolites*, 12(1), Article 60. https://doi.org/10.3390/metabo12010060
14. Melendez, P., Donovan, A., Hernandez, J., Bartolome, J., Risco, C. A., Staples, C. et al. (2003). Milk, plasma, and blood urea nitrogen concentrations, dietary protein, and fertility in dairy cattle. *Journal of the American Veterinary Medical Association*, 223(5), 628–634. https://doi.org/10.2460/javma.2003.223.628
15. Stoop, W.M., Bovenhuis, H., van Arendonk, J. A. (2007). Genetic parameters for milk urea nitrogen in relation to milk production traits. *Journal of Dairy Science*, 90(4), 1981–1986. https://doi.org/10.3168/jds.2006-434
16. Mucha, S., Strandberg, E. (2011). Genetic analysis of milk urea nitrogen and relationships with yield and fertility across lactation. *Journal of Dairy Science*, 94(1), 5665–5672. https://doi.org/10.3168/jds.2010-3916
17. Henao-Velásquez, A. F., Múnera-Bedoya, O. D., Herrera, A. C., Agudelo-Trujillo, J. H., Cerón-Muñoz, M. F. (2014). Lactose and milk urea nitrogen: Fluctuations during lactation in Holstein cows. *Revista Brasileira de Zootecnia*, 43(9), 479–484. https://doi.org/10.1590/S1516–35982014000900004
18. Huhtanen, P., Cabezas-Garcia, E. H., Krizsan, S. J., Shingfield, K. J. (2015). Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. *Journal of Dairy Science*, 98(5), 3182–3196. https://doi.org/10.3168/jds.2014-8215
19. Guo, K., Russek-Cohen, E., Varner, M. A., Kohn, R. A. (2004). Effects of milk urea nitrogen and other factors on probability of conception of dairy cows. *Journal of Dairy Science*, 87(6), 1878–1885. https://doi.org/10.3168/jds.s0022-0302(04)73346-9
20. Xia, C., Ur Rahman, M. A., Yang, H., Shao, T., Qiu, Q., Su, H. et al. (2018). Effect of increased dietary crude protein levels on production performance, nitrogen utilisation, blood metabolites and ruminal fermentation of Holstein bulls. *Asian-Australasian Journal of Animal Sciences*, 31(10), 1643–1653. https://doi.org/10.5713/ajas.18.0125
21. Huhtanen, P., Hristov, A. N. (2009). A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *Journal of Dairy Science*, 92(7), 3222–3232. https://doi.org/10.3168/jds.2008-1352
22. Broderick, G. A., Faciola, A. P., Armentano, L. E. (2015). Replacing dietary soybean meal with canola meal improves production and efficiency of lactating dairy cows. *Journal of Dairy Science*, 98(8), 5672–5687. https://doi.org/10.3168/jds.2015-9563
23. Hristov, A.N., Heyler, K., Schurman, E., Griswold, K., Topper, P., Hile M. et al. (2015). Case study: Reducing dietary protein decreased the ammonia emitting potential of manure from commercial dairy farms. *The Professional Animal Scientist*, 31(1), 68–79. https://doi.org/10.15232/pas.2014-01360
24. Widyobroto, W. P., Rochijan, R., Ismaya, I., Adiarto, A., Suranindyah, Y. Y. (2016). The impact of balanced energy and protein supplementation to milk production and quality in early lactating dairy cows. *Journal of the Indonesian Tropical Animal Agriculture*, 41(2), 83–90. https://doi.org/10.14710/jitaa.41.2.83-90
25. Schöbitz, J., Albarrán, M. R., Balocchi, O. A., Wittwer, F., Noro, M., Pulido, R. G. (2013). Effect of increasing pasture allowance and concentrate supplementation on animal performance and microbial protein synthesis in dairy cows. *Archivos de Medicina Veterinaria*, 45(3), 247–258. http://doi.org/10.4067/S0301-732X2013000300004
26. Zhao, X., Zheng, N., Zhang, Y., Wang, J. (2025). The role of milk urea nitrogen in nutritional assessment and its relationship with phenotype of dairy cows: A review. *Animal Nutrition*, 20(1), 33–41. https://doi.org/10.1016/j.aninu.2024.08.007
27. Roy, B., Brahma., Ghosh, S., Pankaj, P. K., Mandal, G. (2011). Evaluation of milk urea concentration as useful indicator for dairy herd management: A review. *Asian Journal of Animal and Veterinary Advances*, 6(1), 1–19. http://doi.org/10.3923/ajava.2011.1.19
28. Yoon, J. T., Lee, J. H., Kim, C. K., Chung, Y. C., Kim, C.-H. (2004). Effect of milk production, season, parity and lactation period on variations of milk urea nitrogen concentrations and milk components of Holstein dairy cows. *Asian-Australasian Journal of Animal Sciences*, 17(4), 479–484. http://doi.org/10.5713/ajas.2004.479
29. Guinn, J. M., Nolan, D. T., Krawczel, P. D., Petersson-Wolfe, C. S., Pighetti, G. M., Stone, A. E. et al. (2019). Comparing dairy farm milk yield and components, somatic cell score, and reproductive performance among United States regions using summer to winter ratios. *Journal of Dairy Science*, 102(12), 11777–11785. https://doi.org/10.3168/jds.2018-16170
30. Elseed, A. M. A. F., Nada, Mohammed, A., Elmanan, B. A. A. (2014). Milk urea nitrogen as a monitoring tool for assesing protein nutritional status of lactating dairy cows in Khartoum North Sudan. *International Journal of Development and Sustainability*, 3(4), 917–922.
31. Gulinski, P., Ewa, S., Krystof, M. (2016). Improving nitrogen use efficiency of dairy cows in relation to urea in milk — A review. *Animal Science Paper and Reports*, 34(1), 5–24.
32. Horký, P. (2014). Effect of protein concentrate supplement on the qualitative and quantitative parameters of milk from dairy cows in organic farming. *Annals of Animal Science*, 14(2), 341–352. https://doi.org/10.2478/aoas-2014-0008
33. Zhang, J., Zheng, N., Shen, W., Zhao, S., Wang, J. (2020). Synchrony degree of dietary energy and nitrogen release influences microbial community, fermentation, and protein synthesis in a rumen simulation system. *Microorganisms*, 8(2), Article 231. https://doi.org/10.3390/microorganisms8020231
34. Castillo, A.R., Kebreab, E., Beever, D.E., France, J. A. (2000). A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. *Journal of Animal and Feed Sciences*, 9(1), 1–32. https://doi.org/10.22358/jafs/68025/2000
35. Chaveiro, A., Andrade, M., Borba, A. E. S., da Silva, F. M. (2011). Association between plasma and milk urea on the insemination. *Iranian Journal of Applied Animal Science*, 1(4), 221–225.
36. Raboisson, D., Albaaj, A., Nonne, G., Foucras, G. (2017). High urea and pregnancy or conception in dairy cows: A meta-analysis to define the appropriate urea threshold. *Journal of Dairy Science*, 100(9), 7581–7587. https://doi.org/10.3168/jds.2016-12009
37. Tshuma, T., Fosgate, G., Webb, E., Swanepoel, C., Holm, D. (2023). Effect of temperature and humidity on milk urea nitrogen concentration. *Animals*, 13(2), Article 295. https://doi.org/10.3390/ani13020295
38. Consentini, C. E. C., Souza, A. H., Sartori, R., Carvalho, P. D., Shaver, R., Wiltbank, M. C. (2023). Relationships among total mixed ration nutritional components and reproductive performance in high-producing dairy herds. *JDS Communications*, 4(2), 138–143. https://doi.org/10.3168/jdsc.2022-0265
39. Mesen, T. B., Young, S. L. (2015). Progesterone and the luteal phase: a requisite to reproduction. *Obstetrics and Gynecology Clinics of North America*, 42(1), 135–151. https://doi.org/10.1016/j.ogc.2014.10.003

40. Bindari, Y. R., Shrestha, S., Shresthal, N., Gaire, T. N. (2013). Effects of nutrition on reproduction — A review. *Advances in Applied Science Research*, 4(1), 421–429.
41. Silva, A. L., Detmann, E., Dijkstra, J., Pedroso, A. M., Silva, L. H. P., Machado, A. F. et al. (2018). Effects of rumen-undegradable protein on intake, performance, and mammary gland development in prepubertal and pubertal dairy heifers. *Journal of Dairy Science*, 101(7), 5991–6001. https://doi.org/10.3168/jds.2017-13230
42. Xu, L., Yuan, Y., Che, Z., Tan, X., Wu, B., Wang, C., Xu, C., Xiao, J. (2022). The hepatoprotective and hepatotoxic roles of sex and sex-related hormones. *Frontiers in Immunology*, 13(1), Article 939631. https://doi.org/10.3389/fimmu.2022.939631
43. Ipsa, E., Cruzat, V. F., Kagize, J. N., Yovich, J. L., Keane, K. N. (2019). Growth hormone and insulin-like growth factor action in reproductive tissues. *Frontiers in Endocrinology*, 10(1), Article 777. https://doi.org/10.3389/fendo.2019.00777
44. Xu, X.-L., Huang, Z.-Y., Yu, K., Li, J., Fu, X.-W., Deng, S.-L. (2022). Estrogen biosynthesis and signal transduction in ovarian disease. *Frontiers in Endocrinology*, 13(1), Article 827032. https://doi.org/10.3389/fendo.2022.827032

AUTHORS INFORMATION

Sri Mulyati, Ph.D., Lecturer, Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62–811–3251–106, E-mail: sri-m@fkh.unair.ac.id
ORCID: https://orcid.org/0000-0002-0193-4898

Fachruddin Aziz, M. Sc., Student, Master Program of Reproductive Biology, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62–856–4003–1825,
E-mail: azizfachruddin14@gmail.com
ORCID: https://orcid.org/0009-0000-8949-485X

Aswin R. Khairullah, Ph.D., Researcher, Research Center for Veterinary Science, National Research and Innovation Agency (BRIN). Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia. Tel.: +62–814–5510–7656,
E-mail: aswinrafif@gmail.com
ORCID: https://orcid.org/0000-0001-9421-9342

Imam Mustofa, Professor, Lecturer, Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62–812–3561–540,
E-mail: imam.mustofa@fkh.unair.ac.id
ORCID: https://orcid.org/0000-0003-4543-1659
* corresponding author

Budi Utomo, Professor, Lecturer, Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62–815–5316–0631,
E-mail: budi-u-2@fkh.unair.ac.id
ORCID: https://orcid.org/0000-0002-1147-3263

Pudji Srianto, Professor, Lecturer, Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62–896–6504–0661,
E-mail: pudji-s@fkh.unair.ac.id
ORCID: https://orcid.org/0000-0002-6832-9799

Tri W. Suprayogi, Professor, Lecturer, Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62–813–3021–5592,
E-mail: tri-w-s@fkh.unair.ac.id
ORCID: https://orcid.org/0000-0002-2366-5027

Suzanita Utama, Ph.D., Lecturer, Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62–856–3253–815,
E-mail: suzanita-u@fkh.unair.ac.id
ORCID: https://orcid.org/0000-0003-3907-5969

Wurlina Wurlina, Professor, Lecturer, Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62–812–3008–090,
E-mail: wurlina@fkh.unair.ac.id
ORCID: https://orcid.org/0000-0002-1307-3845

Arif N. M. Ansori, Ph.D., Researcher, Postgraduate School, Universitas Airlangga. Kampus B Dharmawangsa, Jl. Airlangga 4–6, Surabaya, East Java 60286, Indonesia; Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University. Chakrata Rd, Dehradun, Uttarakhand 248007, India; Medical Biotechnology Research Group, Virtual Research Center for Bioinformatics and Biotechnology. Jl. Gunung Anyar Kidul No. 33, Surabaya, East Java 60493, Indonesia. Tel.: +62–821–4464–7832,
E-mail: ansori.anm@gmail.com
ORCID: https://orcid.org/0000-0002-1279-3904

Adeyinka O. Akintunde, Ph.D., Lecturer, Department of Agriculture and Industrial Technology, Babcock University. Ilishan-Remo 121103, Nigeria. Tel.: +234–803–478–4244, E-mail: adeyinka.akintunde@gmail.com
ORCID: https://orcid.org/0000-0002-6013-0902

Chairdin D. Nugraha, Ph.D., Researcher, Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN). Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia. Tel.: +62–852–0456–3939,
E-mail: chairdin@gmail.com
ORCID: https://orcid.org/0000-0001-8346-5468

Riza Z. Ahmad, Ph.D., Researcher, Research Center for Veterinary Science, National Research and Innovation Agency (BRIN). Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia. Tel.: +62–817–4922–649, E-mail: riza011@brin.go.id
ORCID: https://orcid.org/0000-0002-2067-717X

Lili Anggraini, Ph.D., Researcher, Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN). Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia. Tel.: +62–852–7465–7948,
E-mail: lilianggraini202403@gmail.com
ORCID: https://orcid.org/0000-0003-3492-292X

Latifah Latifah, Ph.D., Researcher, Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN). Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia. Tel.: +62–851–5923–6299,
E-mail: latifah.sahababintang@gmail.com
ORCID: https://orcid.org/0009-0007-4692-5008

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

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