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Influence of dietary energy levels and protein degradability ratios on reproductive performance and serum mineral profile in early lactating cows

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Abstract

This study evaluated the effects of three total mixed rations (TMRs) with varying energy levels and RDP:RUP ratios on reproductive performance and mineral status in early lactating crossbred dairy cows over 90days. Twenty-one cows were randomly assigned to three isonitrogenous dietary treatments: G1 (60% TDN, RDP:RUP ratio 70:30), G2 (65% TDN, RDP:RUP ratio 70:30), and G3 (70% TDN, RDP:RUP ratio 60:40). Parameters assessed included days in milk at first artificial insemination, service period, pregnancy rate and serum mineral concentrations. While no significant differences were observed among the groups, the results indicated that all diets adequately supported reproductive performance and maintained mineral balance in early lactating crossbred cows.

Keywords: *Energy level, RDP: RUP ratio, Reproductive performance, Serum mineral profile*

Nutritional management during early lactation is a key determinant of reproductive efficiency in dairy cows, as energy and protein balance directly influence ovarian function, conception rates, and overall fertility. Negative energy balance (NEB), a common challenge in early lactation, arises when the energy required for milk production exceeds dietary intake, leading to excessive mobilization of body reserves. This energy deficit impairs ovarian function, delays the resumption of cyclicity, and compromises fertility (Roche, 2006). Restricted energy intake before calving has also been associated with prolonged calving intervals, increased days open, and reduced pregnancy rates following artificial insemination. Conversely, excessive dietary energy intake can lead to metabolic disorders such as hepatic lipidosis and suppressed ovarian function, further deteriorating reproductive performance (Desta, 2024).

Protein nutrition also plays a crucial role in fertility, with its effects largely dependent on the balance between rumen degradable protein (RDP) and rumen undegradable protein (RUP). Protein deficiency is linked to an increased incidence of silent heat, lower first-service conception rates, and reduced overall conception and pregnancy rates compared to cows receiving adequate dietary protein (Salo, 2018). However, excessive rumen protein degradation

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results in elevated ammonia and urea levels, which negatively impact fertility. Ammonia disrupts reproductive processes before ovulation, while urea affects post-fertilization events, exacerbates NEB, delays the onset of cyclicity, and lowers uterine pH, impairing follicular development and embryonic growth (Tamminga, 2006). Additionally, imbalances in energy or protein can disrupt mineral absorption and utilisation, further compounding reproductive inefficiencies during early lactation (Roche, 2006).

Despite extensive research on the individual effects of energy and protein on reproduction, limited studies have explored their combined influence on reproductive efficiency in dairy cows. This study aims to evaluate the impact of three total mixed rations (TMRs) with varying energy levels and RDP:RUP ratios on reproductive performance and serum mineral profiles in early lactating crossbred dairy cows. The study was conducted on 21 early lactating crossbred dairy cows at the KVASU livestock farm, randomly assigned to three dietary treatments (n = 7). The cows were managed under uniform environmental and housing conditions and fed isonitrogenous (13% CP) straw-based TMRs with varying energy levels and RDP:RUP ratios: G1 (Control): 60% TDN, 70:30 RDP:RUP; G2**:** 65% TDN, 70:30 RDP:RUP; and G3: 70% TDN, 60:40 RDP:RUP. TMRs were prepared in 4 kg blocks and fed three times daily, with feed intake adjusted to maintain orts at 10 per cent of the offered amount. TMRs were analysed for chemical composition following AOAC (2016), while fibre fractions (NDF, ADF) were determined using methods described by Goering and Van Soest (1970) and Van Soest *et al.* (1991). The ingredient and chemical composition of the three experimental TMRs are presented in Table 1 and Table 2. The cost per kg of the TMR was Rs 28/-, Rs 30/- and Rs 33/-, respectively for G1, G2 and G3, respectively. Reproductive performance parameters were recorded, including days to first artificial insemination (AI), service period, number of AI per conception and pregnancy rate. Blood samples were collected towards the end of the trial to analyse serum calcium, phosphorus, magnesium, copper and zinc levels using standard kits. Data were analysed using one-way ANOVA for normally distributed variables, Kruskal-Wallis test for non-parametric data, and chi-square test for pregnancy rates.

The present study evaluated the reproductive performance and serum mineral profile of early lactating dairy cows fed total mixed rations (TMRs) with energy levels of 60, 65 and 70 per cent TDN and RDP:RUP ratios of 70:30, 70:30 and 60:40 in G1, G2 and G3, respectively, while maintaining a uniform crude protein (CP) level of approximately 13 per cent. The days in milk at first artificial insemination (AI) were lowest in G1 (83 days) and highest in G3 (116 days), with G2 (100 days) being intermediate, though the differences were not statistically significant ($p = 0.211$, Table 3)**.** While these values indicate some variability in postpartum ovarian resumption, the lack of significant differences suggests that the dietary modifications in energy and protein balance did not have a pronounced effect on the timing of first AI. Similarly, the service period was shortest in G2 (97 days) compared to G1 (176 days) and G3 (187 days), though the differences were not significant ($p = 0.050$, Table 3), suggesting that moderate energy intake (65% TDN) with a balanced RDP:RUP ratio (70:30) may have provided a more favourable metabolic condition for reproductive recovery. The number of AIs per conception was lowest in G2 (1.33) compared to G1 (3.25) and G3 (3.00), though differences were not statistically significant ($p = 0.056$, Table 3), suggesting that dietary variations did not substantially alter conception success. While previous studies indicate that excess dietary RDP may increase plasma ammonia and urea levels, potentially affecting uterine pH and embryo survival (Tamminga,

Table 1. Ingredient composition of the three experimental TMRs

Ingredients	G1	G2	G3
Maize	13.0	21.0	30.0
Coconut cake	2.5	1.0	8.5
Groundnut cake	4.5	7.5	8.8
Tapioca starch waste	9.00	8.7	10.1
Wheat bran	34.1	22.0	0.5
Vegetable oil	0.3	3.0	5.1
Urea	1.0	1.0	1.0
Calcite	1.6	1.3	0.8
Salt	0.5	0.5	0.5
DCP	0.0	0.5	1.2
Mineral Mix	0.5	0.5	0.5
Paddy straw	33.00	33.00	33.00
TOTAL	100.0	100.0	100.0

Additives (per 100kg): Vitamin supplement 25g (composition per kg: vitamin A- 25 MIU, vitamin D3 - 8 MIU, vitamin E - 16g, vitamin B3 - 26g, vitamin B5 - 16g, vitamin B2- 10g, vitamin K3 - 3g, vitamin B6 - 3g, vitamin B1 - 2g, vitamin B9 -0.5g, biotin - 0.05g, vitamin B12 - 0.016g). Toxin binder - 100g

Table 2. Chemical composition of the three experimental TMRs, % on dry matter basis

Nutrient	G_1	G_2	G_3
Dry matter (% as fed)	94.22	93.10	92.14
Crude Protein	13.54	14.01	13.69
Ether extract	3.12	6.62	8.27
NDF (Exclusive of ash)	39.75	27.01	29.21
ADF (exclusive of ash)	25.59	18.35	21.86
Non-fibre carbohydrate (NFC)*	34.23	44.04	39.89
Total ash	9.37	8.33	8.95
Acid insoluble ash	4.84	3.04	3.40
Calcium	1.56	1.68	1.92
Phosphorus	0.82	0.73	0.97
TDN*	61	65	69
RDP (%CP)*	70	70	60
RUP (%CP)*	30	30	40

*Calculated

Table 3. Reproductive performance of lactating cows on three experimental TMRs

Parameter		G1	G2	G3	*p value*
Days in milk at first AI	Median	83	100	116	0.211
	Mean±SE	105.00 ± 21.85	137.57 ± 25.29	134.00 ± 21.03	
Service period (days)	Median	176	97	187	0.050
	Mean±SE	185.50 ± 28.37	95.33 ± 3.28	206.33 ± 26.10	
No. of AI	Median	3	1	3	0.056
	Mean±SE	3.25 ± 0.25	1.33 ± 0.33	3.00 ± 0.58	
Pregnancy rate	% (no./no.)	57.14 (4/7)	42.86 (3/7)	42.86 (3/7)	0.826

Table 4. Serum mineral concentrations of lactating cows on three experimental TMRs

Parameters	G1	G2	G3	*p value*
Calcium (mg/dL)	11.97 ± 0.20	11.98 ± 0.29	12.13 ± 0.32	0.899
Phosphorus (mg/dL)	5.99 ± 0.15	6.44 ± 0.30	5.93 ± 0.22	0.247
Magnesium (mg/dL)	2.10 ± 0.02	2.09 ± 0.01	2.09 ± 0.01	0.690
Copper (ppm)	0.78 ± 0.03	0.78 ± 0.07	0.73 ± 0.12	0.888
Zinc (ppm)	1.99 ± 0.10	2.05 ± 0.09	2.33 ± 0.18	0.176

Mean ± SE of seven observations

2006), the present findings suggest that the protein-energy ratios used in this study remained within physiologically acceptable limits, preventing significant reproductive impairments. Pregnancy rates were 57.14 per cent in G1 and 42.86 per cent in both G2 and G3, with no significant differences among groups (p = 0.826, Table 3). Although numerically higher pregnancy rates were observed in G1, its prolonged service period and higher AI requirement suggest that conception maintenance may have been less efficient. The similar pregnancy rates in G2 and G3 indicate that the dietary variations in energy and protein balance did not exert a major influence on pregnancy outcomes. Serum mineral concentrations, including calcium, phosphorus, magnesium, copper and zinc, did not show significant differences among the treatment groups (Table 4), indicating that dietary variations in energy level and RDP:RUP ratio did not significantly influence mineral metabolism. Calcium concentrations (11.97–12.13 mg/dL, p = 0.899) remained within physiological limits (Kaneko *et al*., 2008) and were comparable to previous findings (Hasan *et al*., 2021). Phosphorus concentrations (5.93–6.44 mg/dL, p = 0.247) aligned with values reported by Chacko *et. al.* (2017) and Rani (2021), suggesting adequate phosphorus availability across diets. Magnesium levels (2.09–2.10 mg/dL, p = 0.690) were within the normal range (Kaneko *et al*., 2008), with no indication of dietary influence. Copper concentrations (0.73–0.78 ppm, p = 0.888) were consistent across treatments, remaining within the reference range (Yokus and Cakir, 2006; Underwood and Suttle, 1999). Zinc concentrations (1.99–2.33 ppm, p = 0.176) were slightly higher than previous reports (Noaman *et al*., 2012; Rani, 2021) but within broader physiological limits (Spolders *et al*., 2010), suggesting that dietary modifications did not adversely affect mineral metabolism. Since no significant differences were observed in serum mineral levels, it could be inferred that the variations in dietary energy level and RDP:RUP ratio in the present study did not significantly alter mineral absorption or utilisation

The study concluded that TMRs with varying energy levels (60%, 65% and 70% TDN) and RDP:RUP ratios (70:30, 70:30 and 60:40) had no significant effect on reproductive performance or serum mineral profile in early lactating crossbred cows. While G2 (65% TDN, ₹30/kg) showed numerically better reproductive efficiency, the differences were not statistically significant. As no clear reproductive advantage was observed, TMR selection should be based on economic feasibility, with G1 (60% TDN, ₹28/kg) being the most cost-effective option, while G3 (70% TDN, ₹33/kg) incurred the highest cost without additional reproductive benefits.

Summary

This study evaluated the effects of varying energy levels (60%, 65%, and 70% TDN) and RDP:RUP ratios (70:30, 70:30 and 60:40) in total mixed rations (TMRs) on reproductive performance and serum mineral profile in early lactating crossbred cows. The results showed no significant differences in reproductive parameters or serum mineral concentrations, indicating that all TMRs effectively supported fertility and mineral homeostasis. While G2 (65% TDN, ₹30/kg) exhibited numerically better reproductive efficiency, the differences were not statistically significant. Given that G1 (60% TDN, ₹28/kg) was the most cost-effective and G3 (70% TDN, ₹33/kg) incurred higher costs without additional reproductive benefits, G1 may be considered a practical choice for optimising feed cost without compromising reproductive outcomes.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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