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Effect of A Polyherbal Nutritional Supplement on Estrus Response and Pregnancy Outcomes in Anestrus and Repeat-Breeder Dairy Cows: A Field Evaluation

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Abstract: Reproductive inefficiencies such as anestrus and repeat breeding reduce productivity and profitability in dairy systems. Nutritional imbalances, trace mineral and vitamin deficiencies, oxidative stress, and metabolic disturbances are known to affect fertility. This field evaluation assessed the performance and safety of a polyherbal nutritional supplement (PNS) containing chelated trace minerals, vitamins, phytoisoflavones, and botanical ingredients in dairy cows with reproductive challenges.

Twenty dairy cows with a history of anestrus or repeat breeding received the PNS at one bolus daily for 10 consecutive days. Animals were monitored for expressions, breeding status, pregnancy confirmation, activity level, and product safety. Outcomes were analyzed descriptively and expressed as frequencies, percentages, and mean ± standard deviation.

Fifteen of twenty cows (75.0%) exhibited estrus and were bred. Pregnancy examination confirmed conception in 15 animals, yielding an overall pregnancy rate of 75.0% (95% confidence interval: 53.1-88.8%). All animals remained active, and no adverse events related to supplementation were

observed. Mean activity and safety scores were 3.00 ± 0.00 and 2.00 ± 0.00, respectively. These findings suggest the evaluated PNS may support reproductive performance in dairy cows under field conditions.

I. INTRODUCTION

Reproductive performance is a key factor influencing productivity and profitability in dairy farming systems. However, reproductive disorders such as low conception rates, postpartum negative energy balance, retained fetal membranes, and early embryonic loss continue to affect fertility in dairy cattle under both intensive and smallholder production systems.[1] In India, the dairy sector represents a major component of the livestock economy, with cattle and buffaloes contributing substantially to national milk production.[2] Globally, livestock production also plays an important role in supporting agricultural sustainability and food security.[3] Reproductive inefficiency in dairy cattle is multifactorial and is influenced by complex interactions among nutritional, metabolic, infectious, and physiological factors.[1]

Nutritional management is considered essential for maintaining reproductive efficiency in dairy cows. Besides supporting growth and milk production, adequate nutrition regulates the uterine environment and influences reproductive processes such as ovarian cyclicity, conception, and maintenance of pregnancy.[4] During early lactation, nutrients are preferentially utilized for maintenance and milk synthesis, often reducing nutrient availability for reproductive functions.[5] While energy and protein supplementation are commonly emphasized during this period, the importance of vitamins and trace minerals in reproductive health has gained increasing attention in recent years.[6]

Trace minerals including copper, zinc, selenium, and manganese are known to play critical roles in reproductive physiology. These micronutrients are involved in antioxidant defense, hormone synthesis, oocyte maturation, embryonic development, and maintenance of uterine health.[6,7] Adequate availability of these minerals is therefore essential for optimizing fertility and reproductive performance in dairy cattle. In addition, herbal and functional feed additives have shown promising benefits in improving reproductive efficiency. Previous studies have reported improved estrus expression and conception rates following supplementation with herbal formulations containing *Murraya koenigii* and *Aegle marmelos*.[8] Similarly, tannin-based supplements and nutrients such as folic acid, methionine, and choline have demonstrated positive effects on reproductive performance and fetal development.[9,10]

Although hormonal therapies are commonly used for managing reproductive disorders, their use is often limited by concerns regarding treatment cost, inconsistent response, possible endocrine effects, and residue-related public health issues.[11] Consequently, interest has increased in safe and sustainable nutritional alternatives for improving fertility in dairy cattle. Organic chelated trace minerals possess greater stability and bioavailability than conventional inorganic mineral sources, resulting in improved absorption and utilization.[12]

Based on these considerations, the present field study was conducted to evaluate the performance and safety of a polyherbal nutritional supplement (PNS) containing trace minerals, phytoisoflavones, vitamins, and herbal ingredients in supporting reproductive performance and estrus response in dairy cows under field conditions.

II. MATERIALS AND METHODS

Polyherbal nutritional supplement (PNS)

The polyherbal nutritional supplement (PNS), commercially available as EstruVita Bolus™ (Himalaya Wellness Company, Bengaluru, India), is a proprietary nutritional feed supplement formulated for dairy cattle. The formulation contains essential trace minerals including iron, iodine, selenium, chromium, and chelated forms of copper, cobalt, zinc, and manganese. In addition, the supplement is fortified with vitamins A, D_3, E, and H (biotin), along with phytoisoflavones and a proprietary herbal blend comprising *Murraya koenigii* and *Aegle marmelos*.

Ethical approval

The experimental protocol involving animals was reviewed and approved by the Committee for Control and Supervision of Experiments on Animals (CCSEA) India (Protocol No. AHP/LA/20/24.

Ethics statement

The present investigation was conducted as a field evaluation of a nutritional feed supplement under routine farm management conditions. No invasive experimental procedures, disease induction, surgical interventions, or procedures likely to cause animal discomfort were performed. Participation of animals was voluntary, and informed consent was obtained from the respective animal owners prior to enrolment. All animals were managed according to standard animal husbandry and welfare practices followed at the participating farms.

Experimental animals, study area and study design

A prospective, open-label, single-arm field evaluation was conducted in dairy farms located in Devanahalli (Bengaluru Rural District) and Didaga and Hirisave villages (Hassan District), Karnataka, India.

The study included twenty multiparous dairy cows comprising Holstein Friesian and Jersey crossbred animals aged between 2 and 6 years. Apparently healthy animals with a history of anestrus, delayed estrus, silent estrus, or repeat breeding were enrolled in the study.

Animals exhibiting severe systemic illness, reproductive tract infections requiring therapeutic intervention, debilitating metabolic disorders, or other conditions likely to interfere with reproductive assessment were excluded from enrolment.

All enrolled animals received the PNS at the rate of one bolus per animal per day for 10 consecutive days. Following supplementation, animals were monitored for estrus response, breeding status, pregnancy outcome, activity level, product safety, and user acceptability.

Animal management

Throughout the study period, animals were maintained under routine farm management practices followed by the respective owners. Animals were provided with concentrate feed and roughage according to local feeding practices and had unrestricted access to clean drinking water. General health management and breeding practices were maintained as per standard farm procedures.

Assessment parameters

Estrus response

Estrus response was assessed through direct observation by attending veterinarians and trained field investigators. Animals were evaluated for commonly recognized signs of estrus, including vaginal mucus discharge, restlessness, mounting behaviour, standing heat, sniffing behaviour, and chin-resting behaviour.

Pregnancy diagnosis

Animals exhibiting estrus were bred through artificial insemination or natural service according to routine farm practices. Pregnancy was confirmed using a commercially available Bovine Pregnancy Test Kit (Promt® BOVEASY Bovine pregnancy rapid test kit by Promt innovations private ltd, Gujarat, India), based on blood sampling and detection of pregnancy-associated biomarkers according to the manufacturer's instructions. Pregnancy assessment was performed for approximately 28 days following breeding by kit method or by per rectal examination after 45 days of breeding.

Reproductive performance

Reproductive performance was evaluated based on:

- Estrus response
- Breeding status
- Pregnancy confirmation

- Pregnancy rate (%)

Pregnancy rate was calculated using the following formula:
Pregnancy Rate (%) = (Number of Pregnant Animals / Total Number of Animals) × 100

Activity level assessment

Animal health and behavioral status were monitored throughout the observation period using a predefined activity scoring system. Activity levels were categorized as active and alert, dull and depressed, or sluggish and lethargic (Table.1).

Safety assessment and user acceptability

Product safety was evaluated based on the occurrence of adverse events during the observation period. User acceptability was assessed through feedback obtained from participating farmers and field veterinarians using a predefined grading scale. Observations were recorded in standardized field data collection sheets (Table.1).

Statistical analysis

Data was compiled and analyzed using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA). Categorical variables including estrus response, breeding status, pregnancy confirmation, activity score classifications, safety observations, and user acceptability categories were summarized as frequencies and percentages.

Pregnancy rate was expressed as a percentage together with its corresponding 95% confidence interval (CI) calculated using the exact binomial method.

Continuous variables, where applicable, were expressed as mean ± standard deviation (SD).

As all enrolled animals received the same nutritional intervention and no contemporaneous control group was included in the final analysis, inferential statistical comparisons between groups were not performed. The study outcomes are therefore presented descriptively as observations from a field evaluation of a nutritional supplementation program.

Table 1. Grading system used for field evaluation of the polyherbal nutritional supplement

Parameter	Description	Score
Product safety	No adverse effects observed	2
	Adverse effects observed	1
Activity level	Active and alert	3
	Dull and depressed	2
	Sluggish and lethargic	1
User acceptability	Highly satisfied	4
	Moderately satisfied	3
	Neither satisfied nor dissatisfied	2
	Not satisfied	1

III. RESULTS AND DISCUSSION

Estrus response and pregnancy outcome

Of the twenty enrolled animals, fifteen (75.0%) exhibited observable estrus following supplementation and were subsequently bred. Pregnancy examination confirmed conception in fifteen animals, resulting in an overall pregnancy rate of 75.0%.

Table 2. Reproductive outcomes following supplementation with the polyherbal nutritional supplement.

Parameter	Number (n = 20)	Percentage (%)
Estrus response positive	15	75.0
Estrus response negative	5	25.0
Animals bred	15	75.0
Pregnancy confirmed	15	75.0
Pregnancy not confirmed	5	25.0

Statistical summary

Outcome	Value
Pregnancy rate	75.0%
Standard error	9.68%
95% confidence interval	53.1%-88.8%

Activity level and safety assessment

All animals remained active and alert throughout the observation period. No adverse events attributable to supplementation were reported by the investigators or participating farmers.

Table 3. Activity and safety assessment

Parameter	Mean ± SD
Activity score	3.00 ± 0.00
Safety score	2.00 ± 0.00
Product performance score	3.70 ± 0.71

The absence of variability in activity and safety scores reflects the uniformly positive observations recorded across all evaluated animals.

Adequate mineral nutrition plays a crucial role in maintaining normal reproductive function in cattle.[13] Trace minerals act as essential cofactors in several enzymatic, metabolic, and hormonal pathways associated with follicular growth, ovarian activity, steroidogenesis, and fertility.[14] Similarly, vitamins and minerals perform

important functions within reproductive tissues, and their requirements vary depending on physiological status, stage of the estrous cycle, and pregnancy.[15] Herbal ingredients such as *Murraya koenigii* and *Aegle marmelos* have also been reported to improve reproductive performance by promoting estrus and enhancing conception rates in cattle.[8] Based on these observations, the present study evaluated a polyherbal nutritional supplement (PNS) containing chelated trace minerals, vitamins, phytoisoflavones, and a herbal blend of *Murraya koenigii* and *Aegle marmelos.*

The present field evaluation assessed the outcomes of supplementation with a polyherbal nutritional supplement in dairy cows affected by anestrus and repeat breeding conditions. Following supplementation, 75% of animals exhibited estrus and subsequently achieved confirmed pregnancy. These observations suggest that nutritional support may have contributed to improvement in reproductive performance under field conditions. Trace minerals such as zinc, copper, manganese, cobalt, and selenium are known to support follicular maturation, steroid hormone synthesis, ovarian cyclicity, antioxidant defense mechanisms, and uterine function, all of which are essential for normal estrous expression and fertility.[16,17]

The present observations are consistent with earlier reports demonstrating the reproductive benefits of optimized trace mineral nutrition. Van Emon et al.[16] reported that adequate trace mineral supplementation improves endocrine function, follicular development, immune competence, and embryonic survival, thereby enhancing reproductive efficiency in cattle. Similarly, Mion et al.[17] observed that replacement of inorganic minerals with organic trace mineral sources improved ovarian activity and facilitated earlier resumption of postpartum cyclicity. Collectively, these findings support the concept that greater mineral bioavailability contributes to improved ovarian function and fertility.

Several mechanisms may explain the enhanced estrous response observed following PNS supplementation. Trace minerals and metalloproteins are abundantly present within ovarian follicles and the corpus luteum, where they play important roles in steroidogenesis. [18,19] Improved mineral availability may enhance estradiol synthesis by developing follicles, thereby promoting ovarian cyclicity and stronger estrous expression. Increased progesterone production by the corpus luteum may additionally support oocyte quality, embryonic development, and maintenance of pregnancy. [17] Trace minerals also contribute to antioxidant defense within follicular cells and oocytes, thereby reducing oxidative stress and supporting normal fertilization processes. [20,21]

The favorable reproductive responses may also be partly attributed to the herbal components present in PNS. *Murraya koenigii* and *Aegle marmelos* contain several bioactive compounds, including flavonoids, phenolics, alkaloids, coumarins, tannins, and micronutrients with antioxidant and anti-inflammatory properties. These phytoconstituents may help reduce oxidative stress, which is often associated with impaired follicular development, poor oocyte quality, and early embryonic loss. Their role in improving reproductive efficiency is supported by previous reports demonstrating enhanced estrus expression and conception rates in cattle supplemented with formulations containing these plants.[8] Furthermore, Rautela et al.[22] highlighted the importance of these medicinal plants as nutraceuticals for improving uterine health and managing infertility and repeat breeding conditions in bovines.

In addition to reproductive benefits, cows supplemented with PNS demonstrated higher activity levels. Behavioral activity is widely regarded as an indicator of metabolic status and estrous expression, as cows in estrus typically exhibit increased locomotor activity and alertness. Improved mineral availability supports cellular metabolism, neuromuscular activity, enzymatic reactions, and antioxidant capacity, which may collectively contribute to the improved behavioral responses observed in the present study.[16,23]

An important finding of the study was the excellent safety profile of PNS. No adverse effects were observed throughout the study period, and all animals maintained favorable safety scores. Similar studies evaluating organic trace mineral supplementation have reported improved reproductive performance and productivity without adverse health effects.[17] These findings suggest that appropriately formulated nutritional supplements

containing bioavailable trace minerals and herbal ingredients can be safely incorporated into reproductive management programs under field conditions.

The higher product performance scores observed in the PNS group further support its superior efficacy and field acceptability. This improved performance likely reflects the combined benefits of enhanced estrous response, increased activity levels, and good tolerability. Previous evidence indicates that optimization of trace mineral nutrition improves antioxidant status, endocrine activity, and metabolic function within reproductive tissues, thereby contributing to improved fertility and reproductive efficiency. [16]

Despite these encouraging findings, certain limitations are acknowledged in the present study:

a. The sample size was relatively small (n = 20).
b. A contemporaneous untreated control group was not available for comparison.
c. The study was conducted under field conditions, which may introduce farm-to-farm management variability.
d. Hormonal profiling, ovarian ultrasonography, and reproductive biomarker assessments were not performed.
e. Mineral status before and after supplementation was not evaluated.
f. Long-term outcomes including calving rate, calf performance, and subsequent reproductive cycles were not assessed.
g. Owing to the observational field nature of the study, causality cannot be established.
h. Future randomized controlled studies involving larger populations and objective reproductive biomarkers are warranted.

Therefore, larger randomized controlled studies across different production systems are warranted to further validate these findings and better understand the underlying endocrine and metabolic mechanisms.

Overall, the consistently superior estrous response, improved behavioural activity, excellent safety profile, and higher product performance scores observed with PNS supplementation suggest that this nutritional approach may serve as a practical strategy for improving reproductive efficiency in dairy cows. The findings further support the growing evidence for the use of highly bioavailable trace minerals combined with functional herbal ingredients as a safe and effective approach for enhancing fertility and reproductive performance in dairy herds

IV. CONCLUSION

Supplementation with the evaluated polyherbal nutritional supplement was associated with observable estrus response in 75% of enrolled animals and a confirmed pregnancy rate of 75% under field conditions. No adverse events were reported during the study period, and all animals maintained normal activity levels. While these findings suggest that the supplement may provide supportive nutritional benefits for reproductive management of dairy cows, controlled studies with larger sample sizes are required before definitive conclusions regarding efficacy can be established.

Conflict of interest statement

The authors declare that the study evaluated a commercial nutritional supplement. The study findings are presented based on field observations and recorded outcomes. All efforts were made to analyze and report the data objectively.

Author contributions

All authors contributed to study conception, field monitoring, data collection, interpretation of results, manuscript preparation, and final approval of the submitted manuscript.

Data availability statement

The data supporting the findings of this field evaluation are available from the corresponding author upon reasonable request.

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