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Research Article

A Field Study to Analyze the Effect of Phytogenics on Performance and Metabolic Health in Transition Dairy Cows

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ABSTRACT

The transition phase for dairy cows is characterized by significant metabolic, hormonal, physiological and immune changes, including negative energy balance, hypocalcemia, liver dysfunction, systemic inflammation and oxidative stress. These disruptions can increase the likelihood of illness, impair reproductive success and negatively impact both milk yield and quality. Recent studies indicate that nutraceuticals-such as probiotics, prebiotics, dietary fats, functional peptides and plant extracts-provide health advantages that go beyond mere nutrition by interacting with various physiological processes. Instead of overlooking systemic inflammatory responses, a more effective strategy involves modulating and quickly resolving these reactions.

Phytoproducts are attracting interest for their wide ranging effects on multiple tissue systems, including antimicrobial, antiviral, antioxidant, immune enhancing, rumen fermentation and microbial modulation properties. This study investigates the efficacy of the herbal preparation RUMEN O, which contains extracts from *Cissus quadrangularis*, *Phyllanthus niruri* and *Terminalia chebula*, in enhancing the health of transition dairy cows. Key health metrics, including feed consumption, negative energy balance, fatty liver incidence, milk fever, postpartum uterine health, retained fetal membranes, metritis and milk production, were monitored. Cows receiving RUMEN O experienced fewer complications and higher milk production, demonstrating the potential of herbal formulations in supporting dairy cow health during the transition period.

Keywords: Phytogenic; Herbal extracts; *Cissus quadrangularis*; *Terminalia*; *Phyllanthus niruri* transition dairy cows; Metabolic health; RUMEN O; Milk production; Negative energy balance; Postpartum complications

INTRODUCTION

Transition period challenges in dairy cows

Around parturition, dairy cows commonly face health issues due to their adjustment from the dry phase to lactation. Research over the latter half of the 20th century and into the present has focused on the "Transition Period" (TP), starting at dry off, exploring its impact on health, immune function and nutrition.

Health problems during the periparturient phase often stem from the cows' struggle to meet the nutritional demands of lactation, leading to physiological imbalances. Such imbalances can heighten the risk of digestive, metabolic and infectious diseases. Impaired immune functions, such as phagocytosis and chemotaxis, increase vulnerability to infections. Nutrition is crucial for immune responses, influencing both directly through nutrients and indirectly *via* bioactive metabolites, particularly in states of physiological imbalance.

The use of nutraceuticals has gained attention for enhancing animal health and productivity. Studies have demonstrated their potential to bolster the immune system and support metabolic activity in critical organs like the liver and mammary gland, particularly during periods of inflammation. These compounds may activate antioxidant defenses and anti-inflammatory pathways, thereby enhancing cell health.

Professor J. Drackley emphasized in 1999 that understanding the biology of the transition to lactation is vital. Research since then

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has shown that immune cells play essential roles in various metabolic functions, including gastrointestinal health and insulin sensitivity regulation. Concurrently, metabolic changes related to energy and calcium supply can impair innate immune responses during TP.

During this period, glucose is prioritized for immune cells and the mammary gland. However, the high glucose demand of an activated immune system during inflammation can exacerbate Negative Energy Balance (NEB), which is prevalent in early lactation. Studies indicate that an activated immune system can utilize over 1 kg of glucose within hours. Severe NEB leads to fat and protein mobilization, resulting in elevated Non-Esterified Fatty Acids (NEFA) in the liver, which can impair its functions [1].

Oxidative stress is another concern during TP, resulting from an imbalance between reactive oxygen species and the body's antioxidant capacity. Increased oxidative stress and inflammation can diminish liver functionality, where Acute Phase Proteins (APP) serve as useful indicators of liver health and inflammation.

Understanding how inflammation in transition cows signals anorexia to the hypothalamus is crucial, as the hypothalamus regulates energy balance by integrating signals from various body regions.

The relationship between rumen fermentation, epithelial adaptations and overall systemic responses remains unclear. Dietary factors, including carbohydrate ferment ability and protein degradability, significantly affect nutrient balance and microbiota composition, impacting rumen health. Thus, exploring feed components that promote rumen stability is essential for enhancing fermentation processes and protecting epithelial integrity [2].

MATERIALS AND METHODS

The study was conducted on a large commercial dairy farm in middle east with a population of transition cows for period of one year and the results were compared with previous years when RUMEN O was not the part of ration. Cows were fed with TMR preparation with RUMEN O added at the rate of 50 grams per cow per day and the cows were monitored for feed consumption, incidence of negative energy balance, fatty liver, milk fever, postpartum uterine health, retained foetal membranes and metritis. Additionally, milk production was tracked throughout the year.

Herbal preparation

RUMEN O was provided by Phyto Specialities Pvt. Ltd. The preparation combines extracts from following three herbs:

Cissus quadrangularis: *Cissus quadrangularis* is a perennial climbing plant belonging to the Vitaceae family, widely found in regions such as India, Sri Lanka, Thailand, Java, West Africa and the Philippines. This vigorous climber can reach heights of up to 11.5 meters, characterized by its quadrangular branches with internodes measuring 8 cm-10 cm in length and 1.2 cm-1.5 cm in width. The plant features toothed green leaves, yellow to red flowers and ripe red globular berries. Commonly known by names such as hadjod, asthisamdhani and devil's backbone, *Cissus quadrangularis* is often referred to as the "bone-setter" due to its applications in orthopedics. It is utilized to promote ossification, enhance fracture healing through osteoblast stimulation, prevent osteoporosis and alleviate pain and inflammation associated with fractures.

Terminalia chebula: *Terminalia chebula*, known as black myrobalan or samor in Thai, is a tree in the Combretaceae family found in tropical and subtropical Asia, including regions like China and Tibet. *Terminalia chebula* contains hydrolysable tannins, primarily gallotannins and ellagitannins, which are oligomeric phenolic compounds with molecular weights ranging from 500 to 3000 D; the highest concentrations are found in the fruit pulp and dried seed pericarp. Gallotannins feature gallic acid esterified with hydroxyl groups of polyol carbohydrates, while ellagitannins form through oxidative linkages in galloyl groups. Additionally, chebulagic acid, a benzopyran tannin, is present in several plant families, with chebulinic acid also found in *T. chebula* fruits. Other phenolic compounds include ellagic acid, gallic acid, tannic acid and chebulic acid, alongside glycosides prevalent in various species. Furthermore, the fruit kernels contain palmitic, stearic, oleic, linoleic and arachidic acids, contributing to the plant's overall activity [3].

Phyllanthus niruri: *Phyllanthus niruri*, a small annual herb from the Euphorbiaceae family, is prevalent in tropical and subtropical regions worldwide. This herb is recognized in traditional medicine for its diverse pharmacological effects, including antimicrobial, antioxidant, anticancer, anti-inflammatory, antiplasmodial, antiviral, diuretic and hepatoprotective properties. *P. niruri* contains a variety of bioactive constituents, such as alkaloids, anthocyanins, chlorogenic acids, coumarins, flavonoids, lignans, phenolic acids, saponins, glycosides, tannins and terpenoids, contributing to its therapeutic potential.

RESULTS

The results indicated a significant reduction in cases of negative energy balance and fatty liver incidences among the cows receiving RUMEN O. Milk production increased by an average of 2.5 liters per cow. Postpartum complications, such as retained fetal membranes, metritis and milk fever, were notably decreased. Specifically, retained fetal membrane cases were reduced by 50% and metritis cases decreased by 33%. Overall, milk production rose by 8.4%, with reproductive performance also improving, evidenced by an 8% increase in conception rates [4].

Feed consumption

Dry matter consumption increased. Cows were fed at 23 kg as fed with 12 kg dry matter in the ration, with other nutrients as per recommended levels. Dry matter consumption increased by 1kg per cows from 11 kg per cow to 12 kg per cow which is 9% increase.

Milk production

The milk production per cow was 26.76 litres per cow, for the period and cows fed without RUMEN O and the average yield per cow was 29.03 for the period and cows fed with RUMEN O. Milk yield increased by 2.27 litres per cow which is 8.5%.

Ketosis and fatty liver cases: Total number of ketosis case incidence reduced significantly and fatty liver cases were not reported during the period of study.

Post-pastum complications

Retained foetal membrane: When the fetal membranes are not expelled within 12 to 24 hours after calving. In this farm all the calved cows are checked for uterine health at 48 hours post calving (Tables 1 and 2).

Table 1: Incidence of retained foetal membranes in cows fed with and without RUMEN O, showing total calvings, number of cases, and corresponding percentages.

Year	No.; Calvings	No.; Retained foetal membrane	Percentage
For the year cows fed with RUMEN O	2813	253	9%
For the year cows fed without RUMEN O	2578	335	13%

For the period of cows fed with RUMEN O there is 4% reduction in retained foetal membrane cases.

Table 2: Incidence of metritis in cows fed with and without RUMEN O, including total calvings, number of metritis cases, and percentage affected.

Year	No.; Calvings	No.; Metritis	Percentage
For the year cows fed with RUMEN O	2813	58	2%
For the year cows fed without RUMEN O	2578	129	5%

For the period of cows fed with RUMEN O there is 3% reduction in metritis cases.

DISCUSSION

Supplementation of RUMEN O containing extracts of *T. chebula*, *Cissus quadrangularis* and *Phyllanthus niruri*, increased overall performances of dairy farm in terms of increased milk production decreased incidences metabolic issues and post-partum complications and increased reproductive performnces of herd by increased the conception rate [5].

Each of ingredients has its bioactive compouds which are responsible for the improved performances of dairy cows.

Cissus quadrangularis

Cissus quadrangularis contains a variety of biologically active phytoconstituents, including steroids, flavonoids, terpenoids, stilbenes, iridoids, tannins and vitamins. Among these, quercetin and resveratrol are the most extensively studied due to their significant therapeutic potential. Catalpol, a prominent iridoid found in CQ, is recognized for its osteopromotive effects. Additionally, CQ comprises numerous enzymes and essential minerals, such as calcium, iron, magnesium, potassium, phosphorus and zinc, which support its diverse biological activities.

CQ has demonstrated promising bone healing properties and has been thoroughly investigated both *in vitro* and *in vivo* for its effectiveness in treating bone disorders and maintaining bone mineral density. The methanolic extract of CQ (CQ-M) exhibits anti-arthritic activity by stabilizing cell membranes, largely attributed to its content of polyphenols, alkaloids, terpenoids and flavonoids. Furthermore, CQ plays a critical role during developmental stages, influencing the differentiation of osteoprogenitor cells and the subsequent formation of bone.

There is potential for CQ to alter estrogen expression in pregnant rats, thereby stimulating osteogenesis in the developing fetus through placental transfer. The enhanced ossification observed in the fetus may be linked to phytosteroids present in the CQ extract, which can act as phytoestrogens. Various fractions derived from crude CQ have been shown to effectively enhance osteoblast differentiation and mineralization, promoting cell attachment, proliferation and mineral deposition.

Mechanism of action of *Cissus quadrangularis*

Cissus quadrangularis contains bioactive compounds that influence bone remodeling by regulating genes involved in osteoblastogenesis (bone formation) and osteoclastogenesis (bone resorption). Key regulators, known as Mitogen-Activated Protein Kinases (MAPKs), mediate the activities of osteoblasts and osteoclasts. Phosphorylation of MAPK in osteoblasts activates Nuclear Factor kappa-B (NFκB), while its

dephosphorylation in osteoclasts prevents the expression of markers like Matrix Metalloproteinases (MMPs), which degrade the extracellular matrix. Thus, MAPK signaling promotes bone formation and inhibits bone resorption.

CQ enhances osteoblast differentiation and mineralization by activating proteins like c-Jun N-Terminal Kinase (JNK) and Extracellular Signal-Regulated Kinase (ERK). Research shows that blocking these proteins reduces Alkaline Phosphatase (ALP) activity, indicating CQ's role in osteoblast regulation.

Flavonoids such as quercetin in CQ may further enhance ALP expression. CQ also stimulates the upregulation of important genes like Runt-Related Transcription Factor 2 (Runx2) and Bone Morphogenetic Proteins (BMPs), promoting bone formation.

Additionally, CQ increases Osteoprotegerin (OPG) levels, which helps regulate osteoclast activity by downregulating RANK and increasing apoptosis in osteoclasts, leading to reduced bone resorption.

Molecular studies have identified quercetin and rutin as key components that boost hydroxyproline, ALP and OPG levels while inhibiting RANKL. Resveratrol, another component in CQ, enhances the OPG

ratio, reducing osteoclast activity and supporting bone health. Its effects, along with those of CQ, play a significant role in bone remodeling [6].

Effect of *Terminalia chebula* Retz., in rumen fermentation, gas production and methane

The pulp contains about 8.4% condensed tannins and 9.9% saponins (dry matter), which may influence rumen fermentation. Patra, et al., found that *T. chebula* pulp could reduce enteric methane emissions in in vitro settings, while also exhibiting antibacterial activity. Supplementation at 0.50 mL led to significant reductions in total protozoa and entodiniomorph counts.

The biologically active secondary metabolites, including total phenolics, condensed tannins and essential oils, can manipulate rumen fermentation. *In vitro* studies by Patra, et al., indicated that *T. chebula* extracts significantly reduced dry matter degradability while improving Neutral Detergent Fiber (NDF) and cellulose digestibility. Additionally, both *T. chebula* and *Allium sativum* showed anti-methanogenic effects alongside improved digestibility. Waghorn and Woodward reported a 15% reduction in methane emissions in sheep and cows fed diets rich in tannin containing forages like lotus. Furthermore, Friesian dairy cows experienced a 23% decrease in methane emissions when consuming *Lotus corniculatus* silage compared to those on ryegrass silage.

Supplementation of *T. chebula* is found to improve rumen fermentation, enhancing propionate concentration, gas kinetics, gas production and reducing both the acetate-to-propionate ratio and methane production.

Hepatoprotective and lipid-lowering effects of *Phyllanthus niruri*

Phyllanthus niruri contains bioactive constituents like phyllanthin and hypophyllanthin, which may protect against Carbon Tetrachloride (CCl_4)-induced cytotoxicity in liver cells. CCl_4 elevates liver injury markers such as glutamate oxaloacetate transaminase and glutamate pyruvate transaminase. Extracts of P. niruri have been shown to significantly reduce these markers without affecting other serum enzymes. An ayurvedic formulation, HPN-12, demonstrated effectiveness in treating liver damage when administered to rats.

A 35-kDa antioxidant protein purified from P. niruri exhibited protective effects against oxidative stress and liver damage caused by various agents, thanks to its free radical scavenging properties.

Amin et al., found that P. niruri could mitigate liver cirrhosis in rats, reducing extracellular matrix synthesis and the expression of transforming growth factor β and collagen markers. Key bioactive constituents, including 4-O-caffeoylquinic acid and quercetin-3-O-rhamnoside, contributed to this hepatoprotective effect [7].

Regarding lipid-lowering properties, P. niruri was studied for its potential in managing hyperlipidemia, which can lead to conditions like acute pancreatitis and atherosclerosis. In experiments with hyperlipidemic rats, the extract lowered serum lipids effectively when administered at 250 mg/kg. Combined administration with cholesterol also reduced lipid and apoprotein levels.

A recent study by Bashir, et al., evaluated the methanolic extract's effect on Glutathione S-Transferase (GST) activity in diabetic rats [8]. While *in vitro* results showed no significant effect, *ex vivo* studies indicated that the extract at 500 mg/kg enhanced GST activity, improving scavenging of free radicals and toxins.

CONCLUSION

The extracts from *Terminalia chebula*, *Cissus quadrangularis* and *Phyllanthus niruri* in RUMEN O demonstrate significant synergistic effects on various physiological processes in dairy cows during the transition period. These bioactive compounds may enhance calcium metabolism and uptake, leading to improved bone mineralization, which is critical for reducing the incidence of milk fever and hypocalcemia. Furthermore, the additive appears to promote smoother muscle contractions, facilitating the unaided expulsion of the fetus and timely removal of fetal membranes.

Terminalia chebula has been shown to positively influence rumen fermentation and microbial health, resulting in increased production of Volatile Fatty Acids (VFAs), which are vital for energy metabolism. Meanwhile, *Phyllanthus niruri* offers protective effects on liver function, contributing to overall metabolic health. Collectively, these effects not only enhance animal performance and productivity but also mitigate complications associated with the transition period.

These synergistic effects of three different extracts might have contributed to the improved performances recorded in terms of milk production, reduced metabolic health issues and post-partum complications.

To fully understand the mechanisms underlying these benefits, further laboratory studies are warranted. Future research should focus on analyzing nitrogen utilization, methane emissions, liver profiles and serum biochemical markers to provide a comprehensive assessment of RUMEN O's effects. Such investigations could lead to optimized feeding strategies and improved health management practices for dairy cows, ultimately benefiting the dairy industry.

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