INFLUENCE OF POLYHERBAL IMMUNOMODULATOR SUPPLEMENTATION ON PRODUCTION PERFORMANCE AND MILK QUALITY OF KARAN-FRIES COWS

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

LIVESTOCK PRODUCTION AND MANAGEMENT

BY

AMIT SHARMA
M.V.Sc. (LPM)

LIVESTOCK PRODUCTION AND MANAGEMENT
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
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Approved By

(EXTERNAL EXAMINER)

(Dr. SHIV PRASAD)
Major Advisor & Chairman (Guide)

Members of Advisory Committee

1. Dr. A.K. Chakravarty
   Principal Scientist, DCB Division
2. Dr. T. K. Mohanty
   Senior Scientist, LPM Division
3. Dr. Sujata Pandita
   Principal Scientist, DCP Division
4. Dr. R. K. Malik
   Principal Scientist, D M Division
5. Dr. B.S. Prakash
   Principal Scientist and Head, DCP Division

CERTIFICATE

This is to certify that the thesis entitled **"INFLUENCE OF POLYHERBAL IMMUNOMODULATOR SUPPLEMENTATION ON PRODUCTION PERFORMANCE AND MILK QUALITY OF KARAN-FRIES COWS"** submitted by **Amit Sharma** towards the partial fulfilment of the requirement for the award of the degree of **Doctor of Philosophy** in **Livestock Production and Management** of the **National Dairy Research Institute (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

Dated: January, 2010

(Shiv Prasad)
Major Advisor & Chairman (Guide)

DEDICATED

TO

LIVESTOCK WELFARE

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Date:

Place: Karnal (Amit Sharma)

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ABBREVIATIONS AND SYMBOLS

@	=	at the rate of
BW	=	Body weight
ad lib	=	ad libitum
ANOVA	=	Analysis of variance
BUN	=	Blood Urea Nitrogen
%	=	Per cent
µg	=	Microgram
µl	=	Microlitre
µmol	=	Micromol
°C	=	Degree centigrade
µg	=	Microgram
DCP	=	Digestible Crude Protein
IU	=	International Unit
Kg	=	Kilogram
mm	=	Millimeter
mmol	=	Milimol
NEFA	=	Non-esterified fatty acids
nm	=	Nanometer
PEI	=	Postpartum estrus interval
pg	=	Picogram
Ppm	=	Parts per million
RFM	=	Retention of foetal membranes
S.E.	=	Standard error
SP	=	Service period
SPC	=	Services per conception
TCA	=	Trichloroacetic acid
TDN	=	Total digestible nutrients
v/v	=	volume by volume
w/v	=	weght by volume
Wt	=	Weight
NEB	=	Negative energy balance

ABSTRACT

The present study was conducted to investigate the effect of polyherbal immunomodulator containing, *Withenia somnifera* (Ashwagandha), *Asparagus racemosus* (Shatavari), *Emblica officinalis* (Amla), *Ocimum sanctum* (Tulsi), *Tinospora cordifolia* (Giloy), *Trebulus terrestris* (Gokhru) *and Nigella sativa* (Klonji) supplementation during peripartum period on subsequent performance of Karan-Fries cows. A total of 40 pregnant Karan-Fries cows in 2-6 parity, on the basis of their expected producing ability, were grouped in to 4 homogenous treatment groups of 10 each. Out of these, one group served as control wherein no supplementation was given. Cows in treatment groups T_1, T_2 and T_3, respectively, were subjected to polyherbal supplementation @ of 150,200 and 250 mg/Kg BW/day from 60 days prepartum to 60 days postpartum.

Immunity status, evaluated on the basis of TLC, DLC, neutrophil phagocytic activity, Plasma micro-minerals (Cu, Fe, Zn and Mn) and IgG concentration and antioxidant status by FRAP and NEFA, in and around calving was significantly better in T_2 and T_3 than control and T_1.

Production performance evaluated on the basis of daily milk, FCMY, fat, protein, lactose and SNF yield during supplementation(1-2 months), residual(3-4 months) and post-residual (5-6 months) period in T_2 and T_3 was significantly higher ($p \leq 0.05$) than control and T_1.

Milk quality in T_2 and T_3, as indicated by lower milk somatic cell and standard plate count was better than control and T_1. This was due to lesser severity and incidence of sub-clinical and clinical mastitis in in T_2 and T_3 treatment groups.

Reproduction performance as indicated by lesser days to postpartum oestrus, first service, number of services/conception, lower service period and higher conception rate in T_2 and T_3 was also better than control and T_1. Economics of milk production indicated that return

from sale of milk over expenditure (supplementation, medication and extra feeding cost due to increased service period) (Rs./cow/day) was highest in T_3 followed by T_2 control and T_1.

Therefore it can be inferred that polyherbal supplementation @ of 200-250 mg/kg BW improved immunity and antioxidant status and thus reduced periparturient stress and associated health problems. This optimized production and reproduction performance and consequently improved economics of milk production.

सारांश

वर्तमान अध्ययन अश्वगंधा, शतावरी, आवंला, तुलसी, गिलोये एवं कलोंजी अ वयवों युक्त बहुपादपीय रोग प्रतिरोधक परिवर्तक के कर्णफ्रिज गायों में ब्यात की अवस्था के आसपास के समय में अनुपूरण का प्रभाव देखने के लिए किया गया । 2 से 6 ब्यात की 40 गायों को उनकी अनुमानित उत्पादन क्षमता एवं ब्यात के अनुसार 10 गायों के 4 समूहों में वर्गीक्रत किया गया । इनमें से एक नियंत्रक समूह की गायों को बहुपादपीय प्रतिरोधक परिवर्तक का अनुपूरण नहीं दिया गया। जबकी समूह 1, 2 व 3 की गायों को प्रसव के 60 दिन पूर्व से 60 दिन बाद तक क्रमशः 150, 200 और 250 मि.ग्रा. प्रति किलोग्राम शारिरिक भार की दर से बहुपादपीय अनुपूरण दिया गया।

रोग प्रतिरोधक क्षमता रक्त के संपूर्ण एवं विभिन्न श्वेत कोशिकाओं की संख्या, उदासीन कोशिका भक्षण क्षमता, प्लाजमा खनिज (तांबा, लोह, जस्ता एवं मैगनिज) सान्द्रता एवं इम्यूनोग्लोबुलिन–जी सान्द्रता के आधार पर एवं प्रति आक्षिकरण अवस्था प्लाजमा एफ.आर.ए.पी. और एन.ई.एफ.ए. के आधार पर नापि आंकी गई जो कि समूह 2 व 3 की गायों में नियंत्रक एवं समूह 1 की गायों के अपेक्षा बेहतर पाई गयी। गायों की उत्पादन क्षमता, दुग्ध, वसा संशोधित दुग्ध, वसा, प्रोटीन, शर्करा एवं वसा रहित ठोस पदार्थ के प्रतिदिन के उत्पादन से आंकी गयी। जो कि बयाहने के 6 महीने बाद तक समूह 2 व 3 की गायों में नियंत्रक एवं समूह 1 की गायों से ज्यादा थी। समू

ह 2 व 3 की गायों के दुग्ध की, दैहिक कोशिकाओं एवं जीवाणुओं की संख्या के आधार पर दुग्ध गुणवत्ता, नियंत्रक एवं समूह 1 की गायों की अपेक्षा अच्छी पायी गय ा। समूह 2 व 3 की गायों म अच्छी दुग्ध गुणवत्ता लक्षण एवं लक्षण रहित थनैला रोग के कम प्रकोप के कारण थी।

नियंत्रक एवं समूह 1 की गायों की अपेक्षा समूह 2 व 3 की गायों ने बयाहने के पश्चात् शीघ्र मद् दर्शाया एवं गर्भधारण किया। समूह 2 व 3 की गायों में गर्भधारण ा दर भी बेहतर पायो गयी।

खर्च के अपेक्षाकृत दुग्ध के बेचने से (रू./ गाय/दिन) अवरोहिक्रम में अधिक लाभ समूह,3, 2, नियंत्रक एवं 1 में पाया गया। इस अध्ययन के परिणामों से यह निष्कर्ष निकलता है कि गायों में बहुपादप युक्त पदार्थ का 200 से 250 मि.ग्रा./कि.ग्रा. शारिरिक भार की दर से अनुपूरण रोग प्रतिरोधक क्षमता एवं प्रति आक्सीकरण अवस्था सुधारकर प्रसव काल के तनाव और संबधित बिमारियों को कम करता है। जो कि गायों की उत्पादन एवं प्रजनन क्षमता बढ़ाने में साहयक है एवं दुग्ध उत्पादन व्यवसाय को अधिक लाभप्रद बनाता है।

Chapter One

Introduction

Chapter One

INTRODUCTION

India has emerged a world leader in milk production and currently, with production capacity of 103 million tones dairy sector is growing at the rate of 4-5 % per annum. Crossbred cows played important role in achieving this goal. This is evident from the fact that despite being only 10% (20.09 million) of cattle population, crossbred cows contribute around 20 % to national milk production pool (BAHS, 2004). Therefore owing to their higher milk production capacity and lower cost of per liter milk production farmers are tempted to rear these crossbred animals (Pathania and Vashist, 2004) but comparative higher susceptibility of these cows to infectious and noninfectious diseases (Jadav et al., 1995, Deshmukh and Kaikini 1999) make the farmers reluctant to rear these cows.

Prevailing intensive animal production system, characterized by continual on farm presence of animals and high stocking density modifies microenvironment in a way which is quite favorable for origin and perpetuation of pathogenic microorganisms. Simultaneously, on the other hand continuous selection for higher milk production has put the cows under constant stress. This stress is more severe during periparturient period.

Periparturient period, which can be defined as 3 weeks before parturition to 3 weeks after parturition (Grummer, 1995) is very critical for the health of cows. During this period cows enter from pregnant non-lactating to non-pregnant lactating state and the cow's

metabolism shifts from the demands of pregnancy to those of lactation, with increased demands for energy and protein. This period is characterized by a substantial decline in feed intake and negative energy and protein balance (Bertics *et al.*, 1992), lipid mobilization leading to elevated plasma NEFA and hepatic triglyceride (TG) content and protein mobilization (Vazquez-Anon *et al.*, 1994). This leads to initiation of chain of physiological and biochemical reactions which causes immunosuppression, which consequently decreases animal resistance to pathogenic microorganisms. Therefore increased challenge of pathogenic microorganisms and decreased animal resistance renders animals more susceptible to various infectious and non-infectious diseases during periparturient period (Goff, 2000). These diseases have considerable negative impact on profitability of dairy farming and jeopardize the success of dairy farm. Economic losses on account of diseases mainly occur due to mortality, morbidity, suboptimal production and reproduction performance decreased functional life and treatment cost.

Among the various diseases, Mastitis, an inflammation of udder, is most important and a major concern for dairy farmers in India and across the world. Mastitis is polymicrobial in origin and characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissue (Blood, *et al.* 2000). In India, the annual economic loss only on account of mastitis to the country's dairy sector is reckoned at nearly over Rs. 6,000 crores. The losses on account of subclinical mastitis (Rs. 4400 crores) are much higher than clinical mastitis (Rs. 1700 crores) (Business line, 2002). Clinical mastitis affects roughly 10 percent of country's milch animal population of six crores cows and four crores buffaloes (Burman 2002). In India, the average incidence of sub clinical and clinical mastitis has been reported as 24.40 and 43.9 % in buffaloes and

24.75 and 54.74% in cows respectively (Hogberg and Lind, 2003). In addition to periparturient disorders, incidence of mastitis during peripartum period is much higher than other period. Low plasma concentration of α- tocoferol at parturition is a significant risk factor for intramammary infection and mastitis during the first week of lactation (Weiss *et al.*, 1997). About 18-40% of cattle and buffaloes are culled primarily due to infertility which incriminates direct losses to the farmer as well as to the genetic resource (Sharma *et al.*, 1993).

Currently antibiotics and other chemotherapeutic agents are being used extensively for the treatment and control of various infectious diseases. But this approach is not sustainable due to microbial resistance and associated animal and human health hazards.

Therefore to protect our dairy farmers and to make dairy farming more profitable, a preventive approach at appropriate time is more suitable than control by treatment.

Disease arises when a pathogenic microorganism overcomes the challenges of weak immune system of host. Therefore, strengthening of non-specific immunity of cow can be used as an alternative approach to overcome the high incidence of infectious and non-infectious diseases. Immunomodulators are the substances, either natural or synthetic in origin, which regulates the immune system of the animal in a way, make them capable to fight out stress related ailments and ensure general wellbeing of animals. Effect of Vitamin E and Se (antioxidant) on performance of animals has been extensively studied but little scientific information is available regarding herbal immunomodulators. In ancient Indian literature, plants like *Withenia somnifera, Ocimum sanctum, Tinospora cordfolia, Emblica officinalis, Nigella sativa, Tribulus terrestris and Asparagus racemosus* are reported to have active principles with immunomodulatory,

antioxidant, analgesic, antipyretic and antimicrobial properties. Several *in-vitro* and *in-vivo* experimental studies on laboratory animals and clinical studies in human have been conducted, but scanty scientific information is available regarding the efficacy of herbal immunomodulatory products on productive and reproductive performance of cows and in prevention and control of diseases. Such an alternative approach towards prevention and control of disease in dairy animals will not only solve the problem of antibiotic resistance, drug residues and immunosuppression in human being and animals but also ensure general wellbeing of animals and consequently will increase the functional life of animal. Therefore, present study was planned to address above discussed problems with the following objectives.

1. To evaluate the effect of polyherbal immunomodulator supplementation during peripartum period on subsequent production and reproduction performance

2. To evaluate the effect of polyherbal immunomodulator on immune status of the cows

3. To study the economics of supplementation of polyherbal immunomodulator

Chapter Two

Review of Literature

Chapter Two

REVIEW OF LITERATURE

Optimum production of high quality milk is the core objective for profitable dairy farming. As milk production is closely associated with reproduction performance and sound health of animals. Periparturient period, as associated with reproduction and initiation of milk production, is very critical for the success of dairy farming. Therefore, in order to chalk out proper managemental strategy, literature regarding physiology, associated problems and potential managemental innovations for periparturient cows is comprehensively reviewed under following heads.

2.1 Physio-biochemical Changes in Peripartorient Cow

During periparturient period cow passes through several physiological and biochemical change which are stressful to cows and consequent to depressed immune function and make the cows susceptible to infections.

2.1.1 Haematological Changes

In comparison to postpartum period cows during advance pregnancy has significantly ($p < 0.05$) higher RBCs, PCV, TLC and Hb concentration than the postpartum period and increases towards parturition and decreases away with parturition (Nazifi *et al.*, 2008). Meglia *et al.,* (2001) reported increase in number of leukocytes, neutrophils and monocytes and decrease in number of lymphocytes at the time of calving.

Kornmatitsuk *et al.,* (2001) reported that hemoglobin content increased at the time of calving and TLC and PMN cells were highest at one day after calving whereas mononuclear cells increased 3 days after calving.

Lymphocyte plays important role in mammary gland defense through the secretion of lymphokines and antibodies, which facilitate the destruction of microorganisms (Tragowski, 1983). Lymphocyte proliferation is higher at one week pre- and postpartum than at calving (Daniel *et al.*, 1991) but ability of lymphocytes in response to mitogens to produce antibody around parturition decreases (Smith *et al.*,1985) and (Keherli *et al.* 1989b).

Kehrli *et al.* (1989a, b) reported decreased ability of neutrophils to ingest and kill the bacteria during first week of lactation.

2.1.2 Metabolic Changes

2.1.2.1 Blood glucose

After calving, the initiation of milk synthesis and rapidly increasing milk production greatly demands for glucose for milk lactose synthesis, at a time when feed intake has not reached its

maximum. Because much of the dietary carbohydrate is fermented in the rumen, little glucose is absorbed directly from the digestive tract. Consequently, dairy cows rely almost exclusively on gluconeogenesis from propionate in the liver to meet their glucose requirements. Limited feed intake during the early postpartum period means that supply of propionate for glucose synthesis also is limited. Amino acids from the diet or from skeletal muscle breakdown as well as glycerol from mobilized body fat contribute to glucose synthesis. Glucose supply to the mammary gland is also enhanced by the decreased oxidative use of glucose that accompanies the initiation of lactation (Drackley *et al.*, 2001). In turn, glucose is directed to the mammary gland by the low circulating insulin concentration because, in contrast to adipose and skeletal muscle, mammary uptake of glucose is insulin independent.

During first week after calving, supply of glucose from fermentation of dietary carbohydrates consumed may fall short of glucose demands by as much as 500 g/dl (Drackley *et al.*, 2001). Glucogenic amino acids and glycerol from body fat mobilization likely contribute to making up this shortfall. This results many fold increase in rates of muscle protein mobilization during the first week after calving compared with prepartum values (Overton *et al.*, 1998).

Negative energy balance during early phase of lactation is more intense in cattle than buffalo (Drackley, 1999). Plasma glucose concentrations are lower during the catabolic phase of lactation, and are higher during the anabolic phase of lactation when energy intake is equal or superior to the energy release (Goff, 1999).

Grummer *et al.*, (1995) reported that plasma glucose concentration decreased during the transition period except for a transient increase associated with calving. Hepatic glycogen was reduced and lipid was increased during the transition period. Howes *et al.*, (1963) suggested that increase in protein concentration in cows whose protein requirements had already been met, triggers a more intense gluconeogenesis as depicted by higher glucose levels

2.1.2.2 Blood urea nitrogen

Blood urea concentration is an indicator of energy protein balance (Dhali, 2001; Campanile *et al.*, 1998) and is typically increased in cows deficient in energy. The rise in BUN level may denote imbalance of protein and energy levels in the diet. Increased prepartum protein intake has been reported to cause elevated prepartum plasma urea nitrogen concentration (Campanile *et al.*, 1998; Doepel *et al.*, 2002) while high protein diet fed to postpartum cows has been observed to cause increased blood urea and reduced fertility (Canfield *et al.,* 1990; Roche *et al.*, 2000; Dhali, 2001). Serum BUN levels are influenced not only by renal function but by other physiological and external factors *viz.* Days in milk (Campanile *et al.*, 1997; Grasso *et al.*, 2004), by the diet (Campanile *et al.*, 1997; Dhali *et al.,* 2006) and by season (Qureshi *et al.*, 2002; Dhali *et al.,* 2006).

Rastini *et al.* (2006) reported relationship between BUN and plasma NEFA at 2 weeks postpartum ($p>0.02$). BUN was related with feed efficiency, BW, and postpartum body weight loss. The relationship between BUN and feed efficiency, BW loss, and plasma NEFA suggest energy status of the cow and protein mobilization may be the possible determinants of BUN concentrations, in the first weeks of lactation. There was a tendency for a weak relationship between BUN and feed efficiency, BW, postpartum BW loss, and plasma NEFA at +2 weeks, but not at - 2 weeks and +1 week.

Zebu cattle have better ability to utilize low protein forage as they have a greater ability to reutilize nitrogen. The higher values of serum nitrogen in Zebu cattle may be due to more efficient digestion of dietary proteins (Howes *et al.*, 1963). Wattiaux and Karg, (2004) indicated blood urea nitrogen concentration in 2nd and 3rd week of lactation was associated with feed efficiency, BW, postpartum BW loss and plasma NEFA concentration. Recent evidence linking retained placenta to a malfunction of the immune system (Kimura *et al.*, 2002) suggests that protein nutrition also might impact the incidence of retained placenta.

2.1.2.3. Non - Esterified fatty acids (NEFA)

During periparturient period energy demand of animal increases tremendously as the nutrient requirement of the foetus increases greatly and the body of animal has to be prepared for calving events and for higher milk synthesis just after calving. Grummer *et al.,* (2004) reported that energy requirement for milk production from early lactation to peak lactation exceeds the available energy from feed intake. As a consequence, body reserves are mobilized for milk production demands and to negative energy phase.

Adipose tissue depots in the cow are oriented toward mobilization of NEFA at transition period (McNamara, 1991). Lipogenesis is essentially shut down, and the sensitivity to lipolytic signals (epinephrine and nor-epinephrine) is greatly enhanced in and around calving, particularly one week postcalving (Underwood *et al.*, 2003). Stressors and poor nutritional management that cause decreases in voluntary DMI results in large increases in NEFA immediately after calving. Moreover, factors secreted in response to infection, stress or trauma result in increased NEFA concentration and increased fat in liver (Herdt *et al.*, 1983).

As the concentration of NEFA in blood increases around calving or in early lactation, more NEFA are taken up by the liver (Bell, 1980). Once taken up by the liver, NEFA can be 1) completely oxidized to carbon dioxide to provide energy for the liver, 2) partially oxidized to produce ketone bodies that are released into the blood and serve as fuels for other tissues, or 3) reconverted to storage fat (triglycerides). Ruminants have an inherently low capacity for synthesis and secretion of very-low density lipoproteins (VLDL) to export triglyceride from the liver (Pullen *et al.*, 1989), but a similar capacity to reconvert NEFA back to triglyceride (Graulet *et al.*, 1998). Moreover, the rate of production of triglycerides in the liver is increased at the time of calving (Grum *et al.,* 1996; Litherland *et al.*, 2003).

Cows fed typical diets during the dry period and transition period have an increased concentration of triglyceride in the liver one

day after calving (Skaar *et al.*, 1989; Grum *et al.*, 1996). If NEFA uptake by the liver becomes excessive, fatty liver may develop. Nyman *et al.,* (2008) investigated association between serum concentration of several blood variables related to metabolic and immunological status around calving. They observed that higher concentration of β-hydroxybutyrate and glucose before calving were associated with lesser SCC at first test milking, whereas higher concentration of NEFA before calving was associated with higher SCC counts at first test milking. NEFA testing during the last days of a dry period is a reliable predictor of fat mobilization and energy status during this transition period (Drackley, 1999).

Grummer *et al.*, (1995) reported that pregnancy decreased feed intake during late gestation, lactogenesis, and parturition have dramatic effects on metabolism in dairy cows during the transition period from 3 weeks before calving to 3 weeks after calving. Increases in plasma NEFA occur during the 10 days before calving and may precede the decrease in feed intake. Plasma NEFA concentrations were highest at calving and decreased rapidly after calving. Fronk *et al.,* (1980) reported that over conditioned cows may be more susceptible to a prepartum decrease in feed intake. Increasing nutrient density of the diet during the transition period may enhance feed intake. Feeding more fermentable carbohydrate during the prepartum transition period may acclimatize the microbial population to lactation diets, promote development of ruminal papillae, increase absorptive capacity of the rumen epithelium, and reduce lipolysis by delivering more glucogenic precursor to the liver and enhancing blood insulin. Drackley, (1981) reported that supplementing fat to transition diets does not seem to alleviate health problems associated with negative energy balance. Enhancing amino acid absorption by the prepartum cow may improve lactation performance and health, although mechanisms of action have not been identified.

Overton, (2000) reported that NEFA concentrations begin to rise during the 7 days before calving, peaked at 3 to 4 days postcalving, and

declined to relatively low levels by 14 to 21 days postcalving. Relationships between metabolites can be used to either troubleshoot transition cow programs, or as a periodic monitor of programs on farms.

Dyk *et al.,* (1995) conducted a large field study (1650 cows) in Michigan, in which blood samples were collected from cows ranging from 35 to 3 days prepartum. Analyses for NEFA were conducted, and cows were divided into three groups representing high, medium, or low NEFA concentrations relative to the average NEFA concentration for each day prepartum. Increased concentrations of NEFA prepartum were correlated with all major transition cow metabolic disorders except milk fever. Elevated prepartum NEFA concentrations occur as a result of inadequate energy intake relative to demand; therefore, troubleshooting using NEFA as a tool is relatively straightforward in terms of assessing dry matter intake of close up cows and diet formulation for this group (Doepel *et al.,* 2002).

2.1.2.4 Plasma cholesterol

Cholesterol has been implicated as one possible metabolic mediator of reproduction in lactating dairy cows. Plasma cholesterol concentration significantly increased progressively from 1 to 7 weeks of lactation. Plasma cholesterol concentrations is positively correlated with energy balance, dry matter intake, milk production, and concentrations of urea nitrogen and milk lactose and negatively correlated with milk fat and protein levels (Spicer and Francisco, 2003).

The values of serum cholesterol are usually considered as an indicator of good hepatic lipoproteins production used as carriers of triglycerides, synthesized from NEFA. Total cholesterol indirectly reflect the degree of exogenous energy availability and the hepatic functionality its levels rise because of moderate negative energy balance, lactation, low temperatures and high thermal ranges (Campanile *et al.,* 1994). In a recent study Grasso *et al.*, (2004),

observed a marked effect of calving distance on the plasma cholesterol level (ranging between 2.05 and 3.01 mmol/l). The higher concentration of cholesterol with the advancement of age is probably a physiological adjustment to meet growth requirements. Around parturition, total plasma cholesterol concentration decreases by 20% (Long *et al.*, 1953).

Kweon (1985) during preparturient period, irrespective of milk yield, reported higher disease occurrence rate in cows with low total cholesterol concentration than cows had higher cholesterol level.

2.1.2.5 Micro-minerals (Zn, Cu, Fe, Mn)

Insufficient contents of trace elements in ruminant diets have been related with low disease resistance (Spears, 2000). Several micronutrients such as cobalt (Co), copper (Cu), selenium (Se), and zinc (Zn) have been reported to influence different aspects of the immune system (Paterson & MacPherson, 1990; Reddy & Frey, 1990). As a component of the metalloenzyme Cu-Zn superoxide dismutase (Cu-Zn SOD) stabilizes cell membrane structures, in a similar manner as vitamin E (Reddy & Frey, 1990). As a normal physiologic process, the blood Zn level declines around calving (Goff & Stabel 1990; Xin et al., 1993) due to reduced DMI, transfer of Zn to colostrum and increased stress at this time. Stressors such as parturition and microbial infections decrease the blood Zn concentrations due to redistribution of Zn from blood to tissues, especially the liver (Spears et al., 1991; Underwood & Suttle, 1999).The periparturient stress in dairy cows induces synthesis of metallothionein, a protein associated with Zn metabolism, making Zn less available for bacterial growth (Spears et al., 1991; Xin et al., 1993). Copper is involved in the antioxidant system via its involvement in the enzymes Cu–Zn superoxide dismutase (SOD) and ceruloplasmin. Copper–Zn SOD is responsible for dismutation of superoxide radicals to hydrogen peroxide in the cytosol (Halliwell and Gutteridge, 1999). Ceruloplasmin

is a Cu transport protein that also exhibits oxidase activity. It oxidizes ferric iron (Fe+3) to ferrous iron (Fe+2) without the production of free Fe+3 that can cause oxidation and peroxidation to tissues (Halliwell and Gutteridge, 1999). Ceruloplasmin is an acute phase protein that increases during disease and may be important in scavenging superoxide radicals (Broadley and Hoover, 1989). Copper deficiency in cattle is generally due to the presence of dietary antagonists, such as sulphur, molybdenum and iron (Fe) that reduce Cu bioavailability (Spears, 2003). Dietary requirements for Cu are greatly increased by high concentrations of molybdenum and sulfur. Considerable research has indicated that dietary Cu affects phagocytic as well as specific immune function (Spears, 2000; Weiss and Spears, 2006). However, limited research with dietary Cu and immunity has been conducted in periparturient dairy cows. Torre *et al.* (1995, 1996) evaluated the effect of dietary Cu on immune function in Holstein heifers. Heifers were fed a control diet containing 6–7 mg Cu/kg diet or the control diet supplemented with 20 mg Cu/kg diet beginning at 84 days pre-partum and continuing into lactation. Neutrophils from heifers fed the low Cu diet exhibited reduced killing of *S. aureus* when blood samples were collected at approximately 35 days post-partum (Torre *et al.*, 1996). Phagocytic activity of neutrophils was not affected by Cu status. Mononuclear cells from heifers were evaluated at approximately 90 days of lactation. Responses of mononuclear cells to mitogen stimulation were not affected by dietary Cu (Torre *et al.*, 1995). However, mononuclear cells from heifers receiving the low Cu diet produced less interferon when stimulated with Con A than cells isolated from cows supplemented with 20 mg Cu/kg diet. Production of IL-2 by mononuclear cells was not affected by dietary Cu. Copper is involved in the antioxidant system via its involvement in the enzymes Cu–Zn superoxide dismutase (SOD) and ceruloplasmin. Copper–Zn SOD is responsible for dismutation of superoxide radicals to hydrogen peroxide in the cytosol (Halliwell and Gutteridge, 1999). Ceruloplasmin is a Cu transport protein that also exhibits oxidase activity. It oxidizes

ferric iron (Fe+3) to ferrous iron (Fe+2) without the production of free Fe+3 that can cause oxidation and peroxidation to tissues (Halliwell and Gutteridge, 1999). Ceruloplasmin is an acute phase protein that increases during disease and may be important in scavenging superoxide radicals (Broadley and Hoover, 1989).

2.2 Periparurient Feed Intake and Performance

Nutritional status of periparturient high yielding cows is the most important determinant of production and reproduction performance in subsequent lactation. As calving approaches, concentrations of progesterone in blood decreases and those of estrogen remain high or increases (Grummer, 1995). The high circulating estrogen is believed to be one important factor that contributes to decreased dry matter intake around calving (Grummer, 1993). During the last 3 wk of pregnancy, nutrient demands by the fetal calf and placenta are at their greatest (Bell, 1980), yet DMI may be decreased by 10 to 30% compared with intake during the early dry period. Negative energy balance and carbohydrate insufficiency in the liver after calving leads to increased production of ketone bodies, which can result in ketosis. Feed intake usually decreases 30 to 35% during the last 3 weeks prepartum while negative energy and protein balance becomes more severe during the week following parturition. Hayirli *et al.* (2002) observed 32 % decreases in DMI, during the final 3 wk of gestation wherein 89% of that decline occurred during the final week of gestation. They also reported that parity, BCS, and concentrations of organic macronutrients in diets affect DMI during the prefresh transition period, and the magnitude of DMI depression as animals approached parturition. DMI was found to decrease linearly with increase in BCS, RUP, and NDF, and quadratically with increase in EE. Increase of RDP was found to cause quadratic increase in DMI.

Doepel *et al.,* (2002) reported that maximizing energy and protein intake during the transition period is paramount to cows making a

smooth transition. Increased energy density of the diet helps combat the drop in feed intake that occurs during the 3 weeks before calving, and helps keep cows in positive energy balance. Poor nutrition during the dry and early postpartum periods has been incriminated for reduced glucose and low LH pulse frequency with concomitant increases in β-hydroxy butyrate, non-esterified fatty acids (NEFA) and triacylglycerol. As a compensatory mechanism cows have been observed to mobilize large lipid and also some protein reserves, resulting in increased incidence of metabolic disorders as hypocalcaemia, acidosis, ketosis, fatty liver and displaced abomasum. Generally negative energy balance has been recognized as a problem of early lactation cows arising from high milk energy output and relatively low feed intake. However, it has, also, been observed a problem for late gestation cows (Grummer *et al.*, 2004) wherein it has been identified as an important predisposing factor for many transition cow disorders such as retained placenta, dystocia, fatty liver and ketosis, reduced feed intake after calving and immunosuppression (Goff, 2000). The disturbed energy balance around calving has been especially associated with periparturient health disorders of dairy cattle (Grummer *et al.*, 2004). Failure to conceive during a restricted breeding season is the most important factor reducing net calf crop. Improved nutrition of the late gestation cow has been postulated to reduce the incidence of some of these disorders (Goff, 2000).

Peripartum disorders have been observed to have a clear correlation with DMI. Dann *et al.* (2005), in ketotic cow reported lower intake, milk yield, and serum glucose concentration but higher serum concentrations of non-esterified fatty acids and β-hydroxybutyrate.

DMI has been observed to have a positive correlation to postpartum reproductive physiology. Cows with higher dry matter intake have been found to be more likely to show signs of estrus at first ovulation and to become pregnant by d 150 of lactation. Increased ratio of plasma glucose to β-hydroxybutyrate has been associated with

a greater probability of estrous expression at first ovulation. Concentration of plasma cholesterol has been reported to be positively associated with expression of estrus at first ovulation, interval from calving to conception, and likelihood of conception and pregnancy. Greater concentrations of non-esterified fatty acids in plasma have been associated with a lower probability of conception by 150 days of lactation (Westwood *et al.,* 2002).

Peripartum energy intake has been recognized as one of the most important nutritional factor affecting production and reproduction performance. Inadequate energy intake in heifers and early lactation cows as well as excessive energy intake during late lactation and the dry period has been associated with alleviated reproductive efficiency. Animals adapt to negative energy balance by mobilizing energy from adipose tissue in the form of NEFA: metabolic and endocrine factors also regulate the rate of NEFA release but with a low degree of sensitivity. Therefore, plasma NEFA concentration reflects the degree of adipose tissue mobilization and negative energy balance. Energy deficiency have been associated with delayed sexual maturity, stop of cycling in heifers and impaired postpartum cyclicity (Beam and Butler, 1999; Opsomer *et al.*, 2000). Negative energy balance (NEB) in the periparturient and early postpartum periods has been regarded as a key risk factor for increased incidence of metabolic disorders. NEB has been associated with greater loss of body condition score and a higher percent of anoestrous cows in the herd and deleterious effects on the follicle or the corpus luteum (CL) by decreasing steroidogenesis (Roche *et al.,* 2000). NEB has been found to affect ovarian function indirectly by decreasing LH-pulsatility and reducing circulating IGF-1, insulin and glucose concentrations (Beam and Butler, 1999) as well as directly on the ovary.

Doepel *et al.* (2002), Overton and Waldron (2004) and Rabelo *et al.* (2005) reported that increased energy supply through dietary carbohydrate during the prepartum period increases plasma glucose

and decreases plasma NEFA concentration on 7 day post calving with positive effects on performance. Cows with the lower energy balance during the far-off period (100NRC and 80NRC) have been reported to have higher dry matter intake and energy balance and lower serum NEFA and ß-hydroxybutyrate during the first 10 days in milk.

2.3 Peripartum Period and Mastitis

2.3.1 Incidence of mastitis

The IDF (1997) has defined mastitis in dairy cows whose cell count of the quarter milk exceeds 500,000cells/ ml. Though, the chances of mastitis are present throughout lactation, but its prevalence is more around parturition and during early dry period. IDF (1997) reported that cows are at higher risk of udder infection on the day of calving, followed by 2 week pre and post partum period. The risk of udder infection during early dry period is comparatively greater than the entire lactation. During peripartum period, several stress factors *viz.* colostrogenesis and calving process contribute to animal's suppressed host defense mechanism. A high proportion of intra-mammary infections (IMI) occur during the first week of dry period when milk flow ceases to flush bacterial invaders from the teat canal before it is fully involuted (Smith, *et al.*, 1985). However, these infections often do not apparent as clinical mastitis, because immune cells eliminate many of these infections in dry period and others are kept in check until lactation begins. These IMI overcome the weak immune system of animal around calving and chances of their conversion into clinical infection increase many folds (Goff, 2000). Besides a week immune system, decline in the antioxidant vitamins in peripartum cows and other physiological factors are also responsible for udder infections.

Around parturition, mammary secretion changes over to colostrum, the level of lactoferrin declines which increases the amount

of iron available for bacterial growth (Goff, 2000). The Keratin plug sealing of teats, breaks down 7-10 days before parturition, permitting easier access to bacteria in the mammary gland (Smith *et al.*, 1985). At parturition, most of the dairy cattle become hypocalcaemic which is suspected to impair smooth muscle contraction, vital for the closure of the teat sphincter after milking. All these factors contribute towards increased incidence of mastitis during periparturient period (Goff 2000).

Cai *et al.* (1994) in a study on dairy cows reported that impaired PMN function was clearly associated with the peripartum metritis and mastitis.

Common stress factors such as parturition, onset of lactation, increased level of steroid hormones, change in feeding and management regimes, depress the immune function and thereby, increase the susceptibility of periparturient cows to infectious diseases (Sordillo *et al.*, 1997).

Klucinski *et al.* (1988) reported that as the lactation proceeds, dairy cows become metabolically stressed due to negative energy balance, leading to mobilization of tissue reserves. This results into increased production of β- hydroxybutyrate and other ketone bodies, which have negative effect on immune function of animals. Hence, the susceptibility to infections is enhanced in and around parturition.

2.3.2. Effect of mastitis on milk yield

Ward and Schultz (1972) observed that milk production starts decreasing when SCC in milk approached $0.5x10^6$/ml. Milk production losses have been reported to be 8, 15 and 27 percent at SCC of $1x10^6$, $2x10^6$ and $4x10^6$/ml milk. Similarly California mastitis test (CMT) score of 1, 2 and 3 corresponded to 10-11, 16-21 and 21-25% decrease in the milk yield of affected animals (Daniel *et al.*, 1966). Jones *et al.* (1984) observed linear decrease in the milk production with increasing somatic cell counts.

Koeldeweij *et al.* (1999) observed that individual milk loss was 1.29 kg/day for each unit of increase in Log_{10} (SCC) for cows in first lactation. Milk yield decreased by 2.04 kg/day per unit log_{10} SCC for older cows. Corresponding loss for protein yield was 0.042 and 0.067 kg/day for first and second lactations.

Horpet *et al.* (1998) used regression models in cows treated for mastitis and calculated loss of 300-400 kg milk/lactation. They further stated that 40% cases were associated with negligible losses, 30% would loose 150-250 kg/lactation and rest 30% lost 950-1050 kg/lactation. Kishk *et al.* (1999) reported 54.7 % loss in milk yield in cows with clinical mastitis.

The decrease in milk production during mastitis is attributed to leucocytic migration into milk which damages the mammary epithelium (Kherli and Shuster, 1994). The neutrophils present in the mastitic gland may release free radicals into the surroundings and thereby destroy the mammary secretary tissue which results into decreased milk production (Ledbetler *et al.*, 2000).

2.3.3 Effect of mastitis on milk Quality

Clinical and sub-clinical mastitis deteriorates milk quality and renders milk unfit for human consumption and further processing for manufacturing various milk products. Mammary gland infection is the single most important factor affecting SCC in milk (Eberhart *et al.*, 1979). As udder infection has the major influence on cell counts, the cell counts in milk could be used as an indirect indicator of the prevalence of infection (Eberhardt *et al.,* 1982).

Elevated somatic cells in milk are associated with various compositional changes in milk (Klei *et al.*, 1998). Harmon (1994) reported change in various milk component (%), in healthy and mastitic milk i.e., fat (3.5 *vs.* 3.2), lactose (4.9 *vs.* 4.4), total

protein (3.61 *vs.* 3.56), total casein (2.8 *vs.* 2.3), whey protein (0.8 *vs.* 1.3), serum albumin (0.02 to 0.07), immunoglobulins (0.1 *vs.* 0.6) and lactoferin (0.02 *vs.* 0.10). Though total protein content had undergone little change, but casein content had decreased significantly. Casein loss into whey increases when milk SCC are above 100,000 cells/ml. Acid degree value of milk increases from 0.27 meq to 0.43meq/100 gm fat as somatic cells increased from 45000 to 84900 (Ma *et al.*, 2000). The explanation for change in milk composition due to increased SCC may be due to injury to udder cells which reduced the synthesis of those milk components which are synthesized in udder such as lactose and most of the casein. The other factor includes the change in permeability of membranes which permit increased leakage of materials from blood to milk. Sodium, chloride and immunoglobulins in milk increased due to this phenomenon (Schultz, 1977). The tyrosine value (TV) which is a measure of proteolytic activity is reported to be high in the milk having SCC above 1.0 million than the milk having SCC of 0.05 million and imparts off flavour to milk and its products (Senyk *et al.*, 1985). Mastitis also decreases shelf life and sensory qualities of milk mostly by higher concentration of free fatty acids. Oxidized flavour in milk is induced with increasing somatic cells in milk (Ma *et al.*, 2000). It has been reported that milk with high SCC had a slower rate of curd formation and produced a softer curd. It is possible that the growth of lactic acid bacteria is inhibited by the antibacterial components present in white blood cells (Guyton, 1971; Pollitis and Ngikwal, 1988). These antibacterial peptides also inhibit starter culture for cheese making (Barbano *et al.*, 1994).

High milk SCC has been linked to impairment in cheese properties and cheese yield potential (Barbano *et al.*, 1991; Auldist *et al.*, 1996). Late lactation milk, which often has elevated SCC, also yields poor quality cheese (Luccey, 1998).

High SCC milk has elevated levels of milk alkaline proteinase plasmin (EC 3.4-21.7), but its activity is not generally considered to be detrimental during cheese ripening (Bastian and Brown 1996). Milk somatic cells are also associated with a number of proteolytic enzymes of differing properties, the activity of which are ill defined in dairy products (Verdi and Barbano, 1991). It may suggest that elevated indigenous proteolytic activity in high SCC milk is due to either plasmin or non plasmin proteinase derived from somatic cells (Klei *et al.*, 1998) or increased plasminogen activation resulting from PMN associated plasminogen activator activity (Heegard *et al.*, 1994 and Cooney *et al.*, 2000).

2.3.4 Effect of mastitis on reproductive performance

Incidence of clinical or sub-clinical mastitis during postpartum period or early lactation period greatly reduces reproductive performance of cows. Barker *et al.* (1998) reported that days to postpartum heat (93.6 *vs.* 71.01), number of AI per conception (2.9*vs.*1.6) were significantly ($p \leq 0.5$) higher for the cows suffered with clinical mastitis before first service than for cows with clinical mastitis after first AI. However, service period (136.6 *vs.* 92.1) days was significantly ($p \leq 0.5$) higher for the cows suffered with clinical mastitis after first service than for the healthy cows or cows suffered with clinical mastitis after pregnancy. They concluded that clinical mastitis during early lactation markedly influenced reproductive performance of Jersey cows.

Clinical and sub-clinical mastitis almost has similar negative effect on reproductive performance of the cows and sub-clinical mastitis followed by clinical mastitis is more detrimental than clinical mastitis alone. Schrick *et al.* (2001) observed no significant difference in days to first service (77.3 *vs.* 74.8), days open (110.0 *vs.* 107.7) and service per conception (2.1 *vs.* 2.1) in cows suffered with clinical and

sub-clinical mastitis during early lactation in Jersey cows. Whereas, days to first service, days open and service per conception in control group were 67.8, 85.4 and 1.6, respectively.

Endotoxin, secreted by Gram negative bacteria induces luteolysis thereby influence conception and early embryonic survival (Culler, 1990).

Inflammatory mediators, in mastitic cow disrupt ovarian function which consequently alters estrous cyclicity (Moore *et al.*, 1991).

Juozaitiene and Juozaitis (2005) studied the relationship between somatic cell count and reproductive performance of Black and white cows and found that the increase of SCC from 1×10^5 to 8×10^5 / ml and over increased the number of insemination per conception from 133.1 to 144.6%, service period from 55.5 to 77.9 % and calving interval from 11.6 to 17.7 % in first three lactations.

2.4 Peripartum Period and Reproduction Disorders

Reproductive disorders are often complex and multi-factorial problems. Among various factors periparturient disorders have been recognized as the most important factors alleviating fertility (Wilde 2006). The occurrence of milk fever and ketosis have been found to affect uterine contractions, delays calving and increases the risk of retained foetal membranes (RFM) and endometritis (Roche, 2006). Preparturient conditions *viz.* hypocalcaemia and ketosis cause parturition associated problems as well as delayed involution of the uterus and metritis (Correa *et al.,* 1993). Therefore, high yielder cows are more susceptible to postpartum reproductive disorders. Deshmukh and Kaikini (1999) and Pandit *et al* (1981) reported that half-breeds were less susceptible to reproductive disorders as compared to 3/4 breeds. Balasundarum (2008) estimated the incidence of reproductive disorder in interbred, F_1 and ¾ groups of Karan-Fries cows and found that the cows in the interbred group were more susceptible to the

reproductive problems. Jadon *et al.* (2005) reported that parturition associated complications increase uterine infections in buffaloes. In 2.73 to 9.72% buffaloes, parturition has been found to be complicated with retained fetal membranes (RFM) (Taraphder, 2002). RFM has been defined as failure to expel fetal membrane within 8-12 hrs of post partum. RFM delay uterine involution, predispose cows to endometritis or metritis and decrease fertility (Grohn and Rajala-Schultz, 2000; Maizon *et al.*, 2004).

2.5 Economic Implications of Periparturient Disorders

2.5.1 Production losses

Next to FMD, mastitis is the costliest disease in dairy industry. As per 1994 estimate, India suffers an annual loss of 1607.2 crores that includes 889.51 crores for cows and 717.69 crores for buffaloes (Singh & Singh, 1994). Dua (2001) reported total economic loss due to clinical and sub-clinical mastitis, Rs. 6053.21 crores per year in India (Table 2.1).

Sasidhar *et al.* (2002) calculated the cost of treating the mastitis cow as Rs. 218.84 which included medicines (41.5%), chemicals (2.71%) and Veterinary aid (55.79%). On an average, the total loss of milk yield was accounted to be 8.90 Kg/day during treatment period and 12.26% losses milk after treatment as compared to before treatment levels. The loss due to SCM is more than CM due to losses in milk production, which is unaccountable for a dairyman. The SCM reduces milk production by 10-25% and also lowers the milk quality (Radostitis *et al.,* 1994). Dhakal (2006) has shown a decrease of 11% in milk yield of mastitis buffaloes resulting in a loss of Rs.4287 per buffalo per lactation. Nearly 68% of the total loss results from drop in milk production. Periparturient disorders and induction of ketosis have been found to have a negative effect on metabolic status and milk yield during the first 14 DIM (Dann *et al.,* 2005). Stillbirth has been associated with decreased milk yield to the extent of 181 kg or 5.2% of

overall mean. Milk yield has been reported to decrease by 239 kg for retained fetal membrane, 173 kg for dystocia, and 98 kg for metritis (Simerl *et al.,* 1992). Taraphder (2002) reported that the overall milk yield and 305 days milk yield were significantly and adversely affected by abnormal calvings in buffaloes.

Table 2.1 Estimated total economic loss due to mastitis in India.

Type	Cows (In Crores)	Buffaloes (In Crores)
Sub-clinical mastitis	Rs. 2646.0	Rs. 1723.32
Clinical mastitis	Rs. 987.6	Rs. 696.29
Total loss	Rs. 3633.6	Rs. 2419.61
Grand total	Rs. 6053.21	

(Dua, 2001)

2.5.2 Reproduction losses

Alleviated reproductive performance has direct and severe economic implications in terms of losses due to reduced production on one hand and additional cost on management on the other (Mulligan *et al.,* 2006). Impairment in the normal reproductive function results into sub-fertility, infertility and sterility, leading to economic losses due to the loss of cycles, extended dry period, delayed maturity, less number of calving and lactations during the lifetime of the animal, increased cost of management and culling due to infertility and sterility (Agarwal and Tomar, 2003; Agarwal *et al.*, 2005).

In cattle, increase in mean calving interval has been incriminated to prolongation of the calving to first insemination interval. In high yielding dairy cows abnormalities in the resumption of post partum ovarian cyclicity have been demonstrated to be as high as 49% (Opsomer *et al.*, 1998) and has been incriminated to no progesterone rise during the first 50 days after calving (delayed cyclicity) or high progesterone levels for more than 20 days without a

preceding insemination (prolonged luteal phase). Such factors have been found to predispose to dystocia and puerperal and other diseases (Opsomer *et al.*, 2000). Losses incurred by these problems being multifaceted are always under estimated.

In one study the expenditure on treatment of abnormal calvings and metritis in buffaloes has been estimated to be Rs 43.61 and Rs 109.43, respectively (Taraphder, 2002). The effect of dystocia has been found to be significant ($P < 0.01$) on all traits within and across parities. Over multiple parities, the differences between score 5 (extreme difficulty) versus score 1 (no problem) for milk yield, fat yield, protein yield, days open, number of services, and cow deaths were 703.6 kg, 24.1 kg, 20.8 kg, 33 d, 0.2 services, and 4.1%, respectively. Across parities, estimates of costs were $0.00, $50.45, $96.48, $159.82, and $379.61 for scores 1 to 5, respectively. However, cost of dystocia has been observed to be relatively higher on a per incidence basis than would be expected from the mean of the population. The total cost associated with dystocia (i.e., within parity sum of costs associated with dystocia scores weighted by the probability of occurrence) has been evaluated to be $28.53 for an average heifer and about $10.00 for an average cow for other parities (Dematawewa and Berger, 1997).

About 18-40% of cattle and buffaloes (Sharma *et al.*, 1993) have been reported to be culled primarily due to infertility which incriminates direct losses to the farmer as well as to the genetic resource.

2.6 Effect of Polyherbal Preparations on Performance of Cows

Anjaria and Gupta (1967) reported that 'Leptaden®' tablet containing shatavari as a major component enhances milk production significantly in buffaloes and cows but not in goat and sheep. Chauhan *et al.* (1971) also reported similar result in crossbred cows.

Arora *et al.* (1983) reported that supplementation of 'Galog®' a herbal preparation having shatavari, significantly enhanced milk production significantly and persistency throughout lactation in the treatment group. Similarly, supplementation of shatavari based herbal formulation 'Payapro®' enhanced milk production significantly. The increase in milk production was on an average 30.85 percent. It has been concluded that herbal preparation showed galactopoietic activity and can be considered as an alternative of lactogenic hormones for inducing and enhancing milk yield in crossbred cows (Singhal, 1995).

Ramesh *et al.* (2000) evaluated the effect of supplementation of 'Galactin®' (50 g /d/animal), a shatavari based polyherbal galactogogue, in lactating crossbred cows and reported significant improvement in milk production over control group. Similarly, supplementation of 'Ruchmax®' increased milk production significantly in buffaloes (Baghel, 2001).

Phalphale *et al.* (1997) reported that herbal preparation 'Ruchmax®' was effective (87.50%) in anorexia in goat and it optimized the activity of the rumen microflora and other ruminal functions, thus, helped to improve the digestion and better utilization of feed by goats.

Chatterjee (1994) studied the effect of herbal preparation immu-21, containing *Ocimum sanctum, Emblica officinalis, Withania Somnifera* and *Tinospora Cordifolia* on the immunological properties in rats and observed increased microbicidal activity of neutrophils and elevated antibody titers in both the primary and secondary immunity assays at dose rate of 20 mg/kg body weight. Cell-mediated immune response was potentiated at lower dose rate (20 mg/kg), but was suppressed at the higher dose (200 mg/kg). They also reported that this herbal preparation protected mice against Escherichia coli lipopolysaccharide-induced mortality.

Das and Chatterjee (1996) found no toxic symptoms like catatonia, disturbances in thermoregulation or abnormal activity in

albino mice supplemented with polyherbal immunomodulator and immunorestorative product at doses of 200 and 400 mg/kg b.w.

Acharya *et al.* (2002) assessed the synergistic effect of polyherbal immunomodulator along with the antibiotic therapy in the treatment of subclinical mastitis in cows. The immunomodulatory effect was evaluated based on the increase in absolute lymphocyte count (ALC) and immunoglobulin G (IgG) level along with clinical recovery. The curative effect was assessed based on the increase in milk yield and reduction in somatic cell count below 0.5 million cells/ml. Significant increases in ALC and IgG were observed in individual cows treated with polyherbal immunomodulators alone and in antibiotic combination. The immunomodulatory effect of the herbal immunomodulator was at par with that of levamisole. Polyherbal immunomodulator alone was found to be effective in 60% of subclinical mastitis cases and it was effective in 100% of the cases when used with antibiotics.

Sahoo *et al.* (2001) evaluated the effect of supplementation of polyherbal immunomodulator (immu-21) to Black Bengal goats, in the last month of the pregnancy and / or kids and reported significantly higher birth weights, increased concentrations of blood protein and colostrum immunoglobulin and absence of kid mortality in the pregnant does and kids supplemented with polyherbal immunomodulator, respectively. They concluded that the supplementation of polyherbal immunomodulator during the later part of pregnancy in goats and to kids during the growth period is much more beneficial than administration at either stage alone. Pradhan and Das (2004) evaluated the therapeutic potential of gentamicin alone and in combination with herbal preparations (Immu-1 and Himax) for the management of goat pox in 50 Ganjam goats with 20% protection level. The results indicated that therapy with Immu-21, gentamicin and Himax ointment was the most efficient (90%), followed by gentamicin and Himax (80%), gentamicin and Immu-21 (70%) and gentamicin alone (50%).

Kolte *et al.* (1999) compared the efficacy of intramammary application of Tilox (ampicillin + cloxacillin; 4 cows with 11 positive quarters) with the topical application of a paste containing roots of *Withania somnifera, Asparagus racemosus and Curcuma amada* and leaves of *Ocimum sanctum* (5 animals with 12 positive quarters) for treatment of subclinical mastitis and found both the preparations effective, as assessed by a return to normal biochemical milk profiles, but the plant preparation acted more slowly.

2.7 Herbs: As Immunomodulators

Several pharmacological, toxicological and clinical studies have been conducted to asses the effect of extracts of *Withania somnifera, Emblica officinalis* (*Phyllanthus emblica*), *Asparagus racemosus, Ocimum sanctum* (*O. tenuiflorum*), *Tinospora cordifolia, Tribulus terrestris* and *Nigella sativa* on various species, which are discussed below.

2.7.1 *Withania somnifera* (Ashwagandha)

The biologically active chemical compounds present in *Withania somnifera* are alkaloids (isopelletierine, anaferine), steroidal lactone (withanolides, withaferins), saponines containing an additional acyl group (sitoindoside VII and VIII) and withanolides with a glucose at carbon 27 (sitoindoside IX and X). Several studies revealed that *Withania somnifera* possess anti-inflammatory properties. Oral administration of powdered root (suspended in 2% of gum acacia) at the rate of 1g/ kg b.w. in rat caused considerable reduction in Freud´s complete adjuvant induced inflammation through significant reduction in acute phase plasma inflammatory proteins *viz.* α 2-glycoprotein, major acute phase α1-protein and pre- albumin (Anbalagan and Sadique, 1981).

The anti-inflammatory effect of *Withania somnifera* is comparable to steroidal anti-inflammatory agents. In Freud´s adjuvant induced paw swelling and degenerative changes in rats, oral

administration of *Withania somnifera* root powder at the rate of 1g/ kg b.w. for 15 days caused significantly ($p \leq 0.05$) higher reduction than hydrocortisone (15 mg / kg) (Begum and Sadique, 1988).

Al-Hindawi *et al.* (1992) in another study also found that *Withania somnifera* inhibited granuloma formation in cotton pelleted implantation in rats and the effect was comparable to hydrocortisone sodium succinate (5 mg / kg). They suggested that anti-inflammatory property of *Withania somnifera* may be due to inhibition of cyclooxygenase enzyme.

Withania somnifera also possess antistress property. This is mainly due to antioxidant activity of glycosides (sitoindosides VII and VIII). Rats supplemented with *Withania somnifera* at the rate of 50 to 100 mg/ kg b.w., exhibited significant antistress activity in forced swimming induced immobility, restraint induced autoanalgia, restraint stress effect on the response of morphine and morphine induced toxicity (Bhattacharya *et al.*, 1987).

Dadkar *et al.* (1987) reported that the alcohol extract of *Withania somnifera* (100 mg/ kg, twice daily orally on day1,4 or 7) reduced stress induced increase in blood urea nitrogen levels, blood lactic acid , and adrenal hypertrophy, but did not affect changes in thymus weight and hyperglycemic rats.

Immunomodulatory property of *Withania somnifera* can be attributed to glycowithanoloides and sitoindosides IX and X. These active principles produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis and increased the activity of the lysosomal enzymes in Swiss mice and Wistar strain albino rats (Ghosal *et al.*1989).

2.7.2 *Emblica officinalis* (Amla)

In addition to high concentration of ascorbic acid, the fruits of *Emblica officinalis* contain phenols (ellagic acid, gallic acid, quercetin, kaempferol, corilagin, greniin and furocin), tannins (gallotanins and emblicanins), flavanoids, glycosides and proanthacyanidines

(Scartezzini *et al.*, 2000; Al- Rehaily *et al.*, 2002 and Zhang *et al.*,2001).

The concentration of phenols in leaves and fruits of *Emblica officinalis* is similar while the roots contain glycosides and tannins (Zhang *et al.*, 2000, 2001).

The ascorbic acid content of fresh fruit juice is very high i.e. 1g/100 ml and it accounts for 45% of the antioxidant activity of *Emblica officinalis* (Scartezzini *et al.*, 2000).

In addition to ascorbic acid, emblicanins, gallic acid, methyl gallate, corilagin furosin and geraniin are other compounds having antioxidant properties (Kumaran *et al.*, 2006).

Emblica officinalis also possess analgesic and antipyretic activity. Alcoholic and aqueous extracts of emblica fruits showed analgesic and antipyretic activity in mice and the effect was comparable to aspirin (Dutta *et al.*, 1998).

In-vitro activity of alcoholic and aqueous extracts of *Emblica officinalis* against certain dermatophytes and common human pathogens supports its anti-microbial property (Ahamad *et al.*, 1998).

2.7.3 *Asparagus racemosus* (Shatavari)

The genus *Asparagus* (Family Asparagaceae, with about 300 species) is a rich source of sapogenins and saponin, from various parts of the plant, (Oketch-Rabah, 1998; Lacaille-Dubois, 2000). The presence of saponin in *shatavari* root has been reported (Jadhav and Bhutani, 2006). Besides saponin, root extract of *shatavari* contain flavonoids (6.7±3.9 mg/100ml), polyphenol including tannin (88.2±9.3 mg/100ml) and Vitamin-C (42.4 ± 5.1 mg/100ml) (Velavan *et al.*, 2007). Mishra *et al.* (2005) reporteded that *shatavari* root contains 4.6 to 6.1% protein, carbohydrates 36.8 to 47.5%, phenols 3.1 to 5.2mg/g, tannins 4.8 to 5.1 mg/g, saponin 4.1% and ash 6.5 to 7.4%. The presence of phyto-components in *shatavari* root such as phytosterols (0.79%), saponin (8.833%), polyphenols (1.692%), flavonoids (0.476%)

and total ascorbic acid (0.762%) were also estimated by Visavadiya and Narasimhacharya, (2007).

Kamat and Venkatachalam (2004) reported that *shatavari* root extract has different types of polysaccharide components (Mol. Wt. 2000 kilodaltons) such as galactose (54%), glucose (28%), Rhamnose (4%), xylose (5%) and arabinose (8%) and others (1%).

Shatavari root contains 91% DM, 8.32% CF, 3.85% CP, 0.66% EE, 74.02% NFE and 13.15% ash (Berhane, 2000). High content of NFE indicates that shatavari is a rich source of energy.

Choudhary and Kar (1992) studied mineral composition and found Ca, Mg, K, and Fe content 0.22, 0.4, 2.5 and 0.005 g/100g, respectively. Whereas, micro-minerals Cu, Zn, Mn, Co and Cr were 5.29, 53.15, 19.98, 22.00 and 1.81 µg/g, respectively. High concentration of microminerals suggests its role in improving reproductive efficiency and immuonopotentiation.

Thatte and Dhanukar (1989) reported that Shatavari supplementation induced leucocytosis with predominant neutrophilia associated with stimulation of phagocytic and bactericidal capacity of neutrophils and macrophages.

Shatavari root has growth promoter property. Calves supplemented with shatavari root decoction at the rate of 100 mg/kg for a varying period of 4 weeks to 8 months showed 81.19 % weight gain as compared to 67.9% in control. It did not have any adverse effect on the progeny of the treated animals. The growth promoting effect can be ascribed to its adaptogenic property (Sharma *et al.*, 1986).

Visavadiya and Narasimhacharya (2005) reported that supplementation of shatavari root powder at 5 and 10% level reduces t plasma and hepatic lipid (cholesterol) levels and also decreases lipid peroxidation.

Barhane and Singh (2002) reported that shatavari supplementation increases dry matter intake significantly in lactating crossbred cows.

Supplementation of shatavari fresh root at the rate of 500 g per day with concentrate at the time of milking significantly increased ($p<0.01$) milk yield of buffaloes (Patel and Kantikar, 1969). Mahantra *et al.* (2003) reported that, feeding herbal formulation containing 25% shatavari enhanced milk production (25.1%) significantly over control group. Somkuwar (2005) and Tanwar *et al.* (2008) reported significant improvement in daily milk yield in buffaloes and crossbred cows, but response of supplementation of shatavari in buffaloes is higher than cows, but the reason was not explained.

The dose of shatavari in dairy animal based on body weight or dry matter intake is not well standardized and supplementation of shatavari @100g/day/animal and 50g/day/animal irrespective of body weight have been found to increase milk production significantly ($P<0.05$) in crossbred cows (Mishra *et al.*, 2008 and Tanwar *et al.*, 2008). However, Berhane and Singh (2002) found that supplementation of shatavari (100gms on alternate day per animal) in freshly calved crossbred cows did not improved milk production. Similar result was also by Vihan *et al.* (1988) in lactating goat.

Berhane, (2000) reported that supplementation of shatavari (100g on alternate day) postpartum alone led to 100% estrus and 75% conception in treatment group as compared to 50% in control crossbred cow within 90 days of calving.

Hegde *et al.* (2002) reported that supplementation of shatavari root powder @ 100g + 10 gm Aloe dried pulp powder to cows following A.I. per animal/day improved conception rate.

2.7.4 *Tinospora Cordifolia* (Giloy)

The active principles of *Tinospora cordifolia* are 20 β-hydroxy-ecdysone, inosporaside, cordioside and columbin (Ahamed *et al.*, 2006). *Tinospora cordifolia* possess antioxidant, hepatoprotective and immunostimulatory properties. Oral administration of aqueous root extract at the rate of 2.5 to 5.0 g/kg BW for 6 weeks in alloxan diabetic

rats decreased plasma thiobarbituric acid, reactive substances, ceruloplasmin and α-tocopherol and increased glutathione and vitamin C concentration. The effect was more than glibenclamide (Prince and Menon, 1999).

Tinospora cordifolia caused significant reduction in serum SGOT, SGPT, ALP, bilirubin and increment in the functional capacities of rat peritoneal macrophages in carbon tetrachloride (CCl_4) intoxicated mature rats. This indicates its hepatoprotective and immunostimulatory functions (Bishayi *et al.*, 2002). They also reported that an alcoholic extract of *Tinospora cordifolia* enhances the differentiation of tumor activated macrophages (TAM) to dendritic cells (DC) in response to granulocyte/macrophage/colony/stimulating factor, interleukin-4 and tumor necrosis factor.

2.7.5 *Ocimum sanctum* (Tulsi)

Fixed oil of *Ocimum sanctum* contains five fatty acids namely, stearic, palmitic, oleic, linoleic and linolenic acids. The pharmacological activity of fixed oil can be attributed to its triglyceride fraction (Singh *et al.*, 1996).

Ocimum sanctum possess hypoglycemic and hypolipidemic properties. Supplementation of tulsi leaf powder at the rate of 1% of feed significant reduced fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglyceride, phospholipids and total lipids in diabetic rats (Ravi *et al.*,1997).

Ocimum sanctum is a potent antioxidant. Alcoholic and aqueous extract of *Ocimum sanctum* at very low concentrations have *in-vitro* anti-lipid peroxidative activity. Aqueous extracts, in a dose-dependent manner in male albino rabbits inhibited hypercholesterolemia-induced erythrocyte lipid peroxidation and also provided significant liver and aortic tissue protection from hypercholesterolemia-induced peroxidative damage (Geetha and Vasudevan, 2004).

Khanna and Bhatia (2003) found that intraperitoneal or oral administration of alcoholic leaf extract of *Ocimum sanctum* at the rate

of 50-100 mg/Kg in mice produced significant analgesia. They also reported that the analgesic action of OS is exerted both centrally as well as peripherally and involves interplay between various neurotransmitter systems.

Ocimum sanctum is a potent immunomodulator and strengthens both humoral and cell mediated immunity. Intraperitoneal injection of *Ocimum sanctum* seed oil at the rate of 3 ml/kg significantly increased anti-sheep RBCs antibody titer and decreased histamine release from peritoneal mast cells of sensitized rats (humoral immune responses) and decreased footpad thickness and percentage leucocyte migration inhibition (LMI) (cell-mediated immune responses). These immunomodulatory effects may be mediated by GABAergic pathways (Mediratta *et al.*, 2002).

Singh (1999) found that fixed oil of *O. basilicum* has significant anti-inflammatory activity against carrageenan and different other mediator-induced paw edema in rats and this is due to blocking of both, cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism.

Singh *et al.* (2005) reported that *Ocimum sanctum* fixed oil showed good antibacterial activity against *Staphylococcus aureus, Bacillus pumilus* and *Pseudomonas aeruginosa*, where *S. aureus* was the most sensitive organism. Higher content of linolenic acid in *O. sanctum* fixed oil could contribute towards its antibacterial activity. The antibacterial activity combined with anti-inflammatory and analgesic activities of the oil could make it useful in inflammatory disorder resulting from *staphylococcal spp.* infection.

Surender *et al.* (1995) compared the efficacy of the fixed oil of *Ocimum sanctum* alone and in combination with cloxacillin sodium in mastitis in buffaloes and reported comparable results with the standard drug (Cloxacillin sodium - 'Floclox - L' injection) used in mastitis. A cure rate of 100% was obtained over a 3- to 5-day period on the basis of quarters treated by intramammary injection with 3.0 ml of fixed oil alone and in combination with 200 mg of cloxacillin sodium.

Reena Mukherjee *et al.* (2005) reported that the aqueous extract of *O. sanctum* treatment reduced the total bacterial count, increased neutrophil and lymphocyte counts with enhanced phagocytic activity and phagocytic index, lysosomal enzymes contents of the milk polymorphonuclear cells (PMNs). The results suggest that the crude aqueous extract of *O. sanctum* (leaf) possesses some biologically active principles that are antibacterial and immunomodulatory in nature. Reena-Mukherjee (2006) reported that the intramammary infusion of aqueous extract of *O. sanctum* reduced the SCC and total bacterial count of *Staphylococcus aureus, Streptococcus spp.* significantly in animals infected with subclinical mastitis.

2.7.6 *Tribulus terrestris* (Gokhru)

The active principles in *T. terrestris* are flavonoid glycosides. These mainly include flavonols, kaempferol, quercetin and isorhamnetin, with the 3-gentiobiosides as the major glycosides (Saleh, 1982).

Amin *et al.* (2006) studied the hepatoprotective effect of *Tribulus terrestris* in streptozotocin induced diabetic rat and reported that oral administration of extract at the rate of 2 g / kg body weight for 30 days, significantly ($P<0.05$) decreased serum ALT and creatinine and incresse in liver GSH enzymes in diabetic rats. Histopathological examination revealed significant recovery of liver in herb-treated rats. This investigation suggested the protective effect in rats may be mediated by inhibiting oxidative stress.

Heidari *et al.* (2007) reported that soxhlet and percolated methanolic extract of *Tribulus terrestris* at the dose of 100 mg/kg produced significant analgesic effect in formalin and tail flick tests in rats. The analgesic effect of the extract was lower than morphine (2.5 mg/kg) in both tests, and higher than aspirin (300 mg/kg) in chronic phase of pain in the formalin test ($P<0.05$). The results of ulcerogenic studies indicate that the gastric ulcerogenecity of plant extract is lower than the indomethacin in the rat stomach.

Singh and Sisodia (1972) reported that extraction of *Tribulus terrestris* fruit powder with water removed the *Cucumis melo* (musk melon) seed produced nephrotoxicity. The washed powder produced a decrease in urinary output, despite increased chloruresis.

Tribulus terrestris also possess antihelmentic property. Alcoholic extract and a mixture of crude alkaloids of the plant *Tribulus terrestris* inhibited the rate and force of contractions of *Ascaridia galli* in vitro, the effects being comparable to those produced by piperazine. The alcoholic extract and alkaloids also eliminated *A. galli* from chickens, although they were less effective than piperazine at similar doses (Chakraborty *et al*; 1979).

2.7.7 *Nigella sativa* (Klonji)

Dadgar *et al.* (2006) reported that ethanolic extract of *Artemisia herba-alba, Nigella sativa, Punica granatum* possessed the most outstanding in vitro antibacterial activity, with maximum inhibition zone of 22.4-18 mm. The lowest MIC value was measured in *Punica granatum* as 0.01 mg/ml against MRSA. The results showed that the ethanolic extract had better antibacterial effect than the aqueous extract and the anti-staphylococcal activity of the ethanolic extract of plants against MRSA was better than MSSA strains.

Tawab and Nasreen (2006) treated Fe (III) solution with extract of *N. sativa* as well as two other active biological reductants, hydroquinone and hydroxyl ammonium chloride and found that *N. sativa* is stronger reducing agent than hydroxyl ammonium chloride and weaker than hydroquinone.

Treatment of the rats with *N. sativa* inhibited ROS production induced by experimental autoimmune encephalomyelitis (EAE) indicated by diminished levels of MDA of both brain and medulla spinalis tissues and NO in brain only. When *N. sativa* was given alone to the rats, no changes were shown in brain, medulla spinalis, and serum oxidant/antioxidant parameters. In conclusion, *N. sativa* may protect brain and medulla spinalis tissues against oxidative stress

induced by EAE. In addition, *N. sativa* display its antioxidant and regulatory effects via inflammatory cells rather than the host tissue (brain and medulla spinalis) for EAE in rats (Ozugurlu *et al*; 2005).

Chapter Three

Materials and Methods

Chapter Three

MATERIALS AND METHODS

The present study was conducted on Karan-Fries cows in the cattle yard at NDRI Karnal, Haryana. The study comprised of two experiments.

3.1 EXPERIMENT 1: EFFECT OF TIME OF OCCURRENCE OF CLINICAL MASTITIS ON PERFORMANCE OF KARAN- FRIES COWS

This study was planned to investigate the incidence of clinical mastitis in relation to time of calving and to compare its impact on production and reproduction performance of Karan-Fries cows. Clinical mastitis incidence data for a period of 10 years (1997-2007) was recorded from mastitis register of animal health complex, NDRI. Affected cows were grouped as follow on the basis of time of onset of clinical mastitis in relation to average service period of herd.

Group1: Cows affected with clinical mastitis before average service period of the herd.

Group 2: Cows affected with clinical mastitis after average service period of the herd.

Reproduction and production performance was compared on the basis of:

1. Service period
2. 305 days milk yield
3. Total Lactation milk yield
4. Lactation length

3.2 EXPERIMENT 2: EFFECT OF POLYHERBA IMMUNOMODULATOR SUPPLEMENTATION DURING PERIPARTUM PERIOD ON PERFORMANCE OF KARAN- FRIES COWS

This experiment was conducted to study the effect of polyherbal immunomodulator supplementation during peripartum period on performance of Karan- Fries cows.

3.2.1 Details of Experiment

3.2.1.1 Procurement and Processing of Herbs

Individual herb was procured from local market after assessing their quality in consultation with ayuverdic practitioner and drug manufacturer. Each herb was pulverized separately. The Polyherbal immunomodulatory preparation was prepared after mixing pulverized herbs in specific proportion. The polyherbal immunomodulatory preparation contained *Emblica officinalis* (Amla), *Asparagus racemosus* (Shatavari), *Withenia somnifera* (Ashwagandha), *Ocimum sanctum* (Tulsi), *Tinospora cordfolia* (Giloy), *Tribulus terrestris* (Gokharu) and *Nigella sativa* (Kalonji).

3.2.1.2 Selection of Experimental Cows

The experiment was carried out on fourty Karan-Fries cows of 2-6 parity. Cows were selected around two months before their expected date of calving. All the cows were free from physiological, anatomical and infectious disorders.

3.2.1.3 Grouping of Experimental Cows

On the basis of lactation number and expected producing ability (EPA), cows were divided in to four groups of ten cows in each so that each group (Table 2.1) had nearly similar average EPA.

Table 3.1 Details of experimental cows

Group	Cow No.	Parity	EPA
Control	KF-6559	3	4724
	KF-6609	3	3997
	KF-6821	2	4091
	KF-6823	2	3945
	KF-6528	4	4593
	KF-6792	2	4213
	KF-6858	2	4357
	KF-6507	4	4834
	KF-6645	2	4075
	KF-6357	5	4834
	Average	**2.9**	**4366.30**
T_1	KF-6761	2	3965
	KF-6768	2	4135
	KF-6659	2	4251
	KF-6778	2	4336
	KF-6610	3	4547
	KF-6543	4	5006
	KF-6773	2	4287
	KF-6845	2	3874
	KF-6892	2	4085
	KF-6377	6	4369
	Average	**2.7**	**4285.50**
T_2	KF-6223	6	4326
	KF-6741	3	3879
	KF-6669	3	4416
	KF-6793	2	4239
	KF-6646	3	4425
	KF-6422	6	3950
	KF-6729	2	4528
	KF-6745	3	4669
	KF-6695	3	4544
	KF-6840	2	3988
	Average	**3.3**	**4296.40**
T_3	KF-6737	2	4590
	KF-6774	2	4312
	KF-6616	4	5255
	KF-6751	3	5007
	KF-6366	5	4534
	KF-6517	3	3784
	KF-6530	4	3384
	KF-6468	4	3828
	KF-6852	2	3953
	KF-6787	2	3980
	Average	**3.0**	**4262.70**

3.2.1.4 Housing Management

Experimental cows were kept in separate barn under loose housing and group management system. The paddocks, in which experimental animals were housed, were large, open and brick on edges flooring. Fifteen days before the expected date of calving, the pregnant cows were shifted to single calving pens provided with ample space, proper ventilation and drainage, soft bedding and feeding and watering facilities. Cows were kept there only during night hours, whereas during day time they were kept in paddock. Each cow was kept in a separate pen in a calving pen. Immediately after calving, the calved cows were taken in to a separate pen attached with court yard, where they were kept individually for a period of five days as per the management practices followed at the institute.

3.2.1.5 Feeding Management

All cows were fed to meet the nutritional requirements as per NRC standards. Daily, green fodder was offered 3 times a day. During experimental period, predominantly green fodder was berseem, lucern, cow pea and maize. Roughage was also offered in limited amount. Total concentrate allowance to pregnant cows was offered in morning while concentrate to lactating cows was offered at the time of milking. Cows had round the clock access to *ad libitum* fresh water.

3.2.1.6 Milking Management

During the first five days of postpartum colostrum period, the cows were hand milked twice in a day by the institute milker at 7.30 AM and 3.30 PM. From sixth day of calving, cows were shifted to normal machine milking shed. Milking was done by milking machine thrice daily (morning, noon and evening). The time of milking was at 4:30AM, 12:30PM and 7:30PM at the Institute milking shed. Before

every milking, lactating cows were cleaned and rinsed properly. The milk yield was recorded in kg with automatic milk gauge.

3.2.1.7 Polyherbal supplementation

Polyherbal preparation was supplemented from 60 days prepartum to 60 days postpartum. Supplementation dose for individual cow was calculated on the basis of fortnightly body weight. Each morning, experimental cows were tied and calculated amount of polyherbal mixture for each cow was offered individually after mixing with 500 g of concentrate. The dose rate for different groups is presented below:

Treatment 1: 150mg / kg BW OD

Treatment 2: 200mg /kg BW OD

Treatment 3: 250 mg/kg BW OD

Control: No supplementation

3.2.2 Production Performance

3.2.2.1 Milk Yield

Daily milk yield of individual cow was recorded. This data was used for calculation of average milk yield of different treatments for different fortnights and periods. Daily milk yield data was also used for calculation of 4% fat corrected milk yield by following formula.

Fat corrected milk yield (Kg) = 0.4 x M + 15 x F

Where,

M= Total milk yield (kg)

F= Total fat produced (kg)

3.2.2.2 Milk Composition

To determine milk composition, about 100 ml of milk sample from individual animal of each milking was collected at fortnightly interval in a properly cleaned milk sample bottle. Finally all three times sample were mixed properly and 50 ml was used for the analysis of milk constituents. The milk constituents such as fat, protein, lactose and SNF were analyzed by Lacto Star- automatic milk analyzer. The total solid values of milk sample were estimated by addition of SNF and fat value.

3.2.3 Udder Health and Milk Quality

Milk quality was assessed fortnightly for following parameters

3.2.3.1 Somatic Cell Count

Somatic cell counts in fresh milk were counted microscopically as per IDF bulletin document no. 321 (1997).

Preparation of Dye

54 ml ethyl alcohol (95%) and 40 ml tetrachlroethane were mixed in a bottle and heated in a water bath at 60-70°C for 15 minutes. Methylene blue was added to the above solution carefully and solution was cooled in refrigeration for 30 minutes. Thereafter, glacial acetic acid (6 ml) was added. Dye solution was filtered using a filter paper with a pore size of 10-12 micron and was kept in air tight bottle.

Method

Milk samples were kept in water bath maintained at 37°C for 5-8 minutes and was mixed carefully. Samples were cooled to room temperature. 0.01 ml of milk was spread evenly in the area of 1.0 cm on a glass slide. Films were dried and put into staining box containing dye solution. After 10 minutes, slides were taken out of the staining box and dried at room temperature. Slides were washed with

tap water until surplus dye was removed. Slides were dried again and stored.

Calculation of Somatic Cells

The number of cells present in each field was counted with the help of a microscope. The diameter of field was measured by using a stage micrometer. The magnification factor (MF) for 1.0 ml of milk was calculated as given below:

$$MF = \frac{100^{(a)} \times 100^{(b)}}{\pi r^2}$$

(a) Area of slide = 1cm = 100mm

(b) Milk = 10 µl= 100

r = radius of field

Average numbers of cells/field were multiplied by MF to obtain total cells in 1 ml of sample.

3.2.3.2 Standard Plate Count

The measurement of standard plate counts of milk samples was performed as per guidelines of ISI (1962), described below. Milk samples were collected from the measuring jar attached to milking machine. Serial 10 fold dilutions of the collected sample were made in phosphate buffer saline or peptone water in sterile test tubes. Aseptically 1 ml of each diluted sample was inoculated into separate pairs of petri dishes. After that 20 ml sterile plate count agar media (Hi-Media) was poured into each of the pair of the petri dishes. By gentle shaking or rotating each of the plate inoculum and the medium was mixed thoroughly. Agar was allowed to solidify and then plates were incubated at 37ºC overnight in inverted manner. After incubation plates were observed for appearance of bacterial colony and selected one plate for each sample containing about 30-300 numbers of

separate colonies of bacteria. Dilution factor of the sample inoculated in the selected plate was recorded.

Number of bacterial colonies per ml = Number of colonies counted x Dilution factor

3.2.3.3 Modified California Mastitis Test (MCMT)

Milk samples were also tested for SCM using MCMT (Sastry, 1978). About 3 ml of milk was collected in a shallow cup after discarding 3-4 streaks of milk from the teat. An equal amount of test reagent (1.5 g NaOH, 0.5 ml teepol and 0.01g bromothymol blue in 100 ml water) was added to the cup and was mixed properly by gentle circular movements of paddle for about 10 sec. Reaction occurred immediately accompanied by precipitation and gel formation in the positive cases due to the presence of leukocytes. Positive milk samples turned greenish blue due to alkalinity. Depending upon the degree of gel formation, the following grades were assigned (Table 3.2).

Table 3.2 MCMT Score for evaluation of SCM in milk

Score	Character	Interpretation
0	No precipitate	Negative
1	Precipitate present but no gel formation	Mild infection
2	Thick precipitate which concentrated towards the center of plastic cups during movement of paddle	Moderate
3	Distinct gel formation which adhered to the bottom of paddle	Severe

3.2.4 Immunity Status

Fortnightly blood samples were collected at -30, -15, 0, 15 and 30 days of parturition to study following parameters.

3.2.4.1 Total leukocyte count (TLC)

The total leukocyte count was made by the Haemocytometer method as described by Schalm (1961). Briefly, blood was drawn up to 0.5 marks in WBC pipette. The end of the pipette was wiped with tissue paper and the diluting fluid was drawn up to 11 mark. The contents of the pipette were mixed thoroughly between palms. Prior to counting, a few drops of the mixture in the stem of pipette were discarded. The Neubauer chamber was charged and the cells were allowed to settle for one minute. The cells were counted under low power in each of the 4 large corner squares. Appropriate dilution factor was used for calculating the total leukocyte count and was expressed as cubic mm.

3.2.4.2 Differential leukocyte counts (DLC)

A thin smear of blood was made on a clear, dry grease free micro aid slides and the smear was dried for one day. Then fixation of the cells on the slides was done by keeping it in methyl alcohol for 5 min. After drying the Field's stain was used for staining slides. Identification of different types of the cell was done by observing the slide under 100 x magnifications under oil immersion by counting 10 fields.

3.2.4.3 Neutrophils Phagocytic Index

Determination of neutrophil phagocytic activity involved following processes.

Separation of neutrophils

Blood neutrophils were separated by the method described by Mehrzad *et al.,* (2004). 15 ml blood samples were taken in heparin containing test tube. Blood samples were centrifuged at 4 ^{0}C at 1000 g for 15 minutes. Supernatant was discarded and 7ml. double distilled water was added and mixed for 45 seconds in remaining packed cell volume. 3 ml. of 2.7% Nacl was mixed for 60 seconds. Centrifuge for 10 minutes at 4 ^{0}C at 1000 g speed. Again supernatant was discarded and 2.5 ml DPBS, 5 ml. DDW and 2.5 ml of 2.7% Nacl was mixed for 60 seconds and centrifuged for 5 minutes at 4 ^{0}C at 1000 g speed. Cell suspension was washed 3 times in PBS and centrifuged at 300 g for 5 minutes at 4 ^{0}C. Final cell suspension was resuspended in 1-2 ml. of PBS with gelatin (0.5 mg/ml). Neutrophils concentration in cell suspension was determined by counting the cells, stained with Field's stain, under oil immersion at 100 x magnifications.

In-vitro Phagocytic activity of blood Neutrophils

Neutrophils Phagocytic activity was measured by modified colorimetric nitro blue tetrazolium reduction assay method as follows.

1. Preparation of RPMI media

Ingredient	Amount
RPMI 1640	16.4 g
$NaHCO_3$	2.1 g
Sodium Pyruvate	10.08 mg
B Mercapto Ethanol	50 µl (1M solution)
HEPES	5.95775 g
Penicillin	61 mg
Streptomycin	100 mg
L glutamine	293 mg
H_2O	1 L

pH was adjusted at 7.4

2. Preparation of zymosan solution

250 µg of zymosan was dissolved in per ml RPMI media.

3. Preparation of NBT solution

600 µg NBT was dissolved in per ml of RPMI media.

4. Culturing of neutrophils

Neutrophils suspension for each sample was cultured in duplicate in ordinary flat bottom plates containing 96 wells as below:

a) Add 10 µl NBT solution in each well used for blank and treatments with the help of micropipette.
b) Add 10 µl zymosan solution added in each well.
c) Add 70 µl of RPMI media, only in blank well.
d) Add a volume of neutrophil cell suspension containing 1x 10^6 cells in treatments well.
e) Add 10 µl of absolute alcohol in blank well.
f) Incubate the plate for 2 hours at 37 ^oC in 5% CO_2 incubator.
g) Measure the optical density of culture media by spectrophotometer at 540 nm wavelength.
h) Take the average of blank and samples reading.
i) Subtract the reading of blank from samples.
j) This optical density denotes phagocytic index, reflecting neutrophil phagocytic activity.

3.2.4.4 Total Plasma Antioxidant Activity

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). FRAP assay uses antioxidants as reluctant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichimetric excess.

Principle

At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) can be monitored by measuring the change in absorption at 593 nm. The reaction is non specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe to Fe^{II}) ion formation. The change in absorbance is, therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture.

Reagents

1. FRAP Reagent

Acetate buffer 3.0 mM, pH 3.6: Weigh 3.1 gm sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1.0 litre with distilled water.

Ferric chloride 2 mM in 40 mM HCl.

Tripyridyl triazine 10 mM

The working FRAP reagent was prepared by mixing A, B & C in the ratio of 10:1:1, at the time of use.

2. Ferrous sulphate 1mM

3. Ascorbic Acid 100 μM

Procedure

Plasma sample (100 μl) was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minute after vortexing. Thereafter, samples were placed at 37°C in water bath and absorbance was measured after 4 minutes. Ascorbic acid standards (100 μM-1000 μM) were processed in the same way.

Sample calculation

Results were calculated as follows.

$$\text{FRAP value of sample (}\mu\text{mol/L)} = \frac{\text{Change in absorbance of sample from 0 to 4 minutes}}{\text{Change in absorbance of standard from 0 to 4 minutes}} \text{ X FRAP value of standard (100}\mu\text{m)}$$

A = Reading of sample at 0 minute

B = Reading of sample at 4 minute

X = Reading of standard at 0 minute

Y = Reading of standard at 4 minute

100 = FRAP value of 100 μM standard

$$\text{FRAP value of sample (}\mu\text{mol/L)} = \frac{\text{A-B}}{\text{X-Y}} \text{ X } 100$$

3.2.4.5 Estimation of Plasma NEFA

NEFA was estimated by copper soap extraction method modified by Ship *et al. (1980).* The method is being discussed under the following headings.

Reagent preparation:

Copper reagent

A mixture of 5ml of triethanolamine and 10ml of 1M aqueous cupric nitrite [Cu $(NO_3)_2.3H_2O$] was diluted to 100ml with saturated sodium chloride solution. The pH was adjusted to 8.3 with 1N sodium hydroxide solution. The mixture was stored in the dark room temperature in order to ensure that the material remained stable for a period of at least 4-5 months.

Colour reagent

0.5% Sodium diethyl dithiocarbamate solution in n-butanol, i.e., 0.5 gm per 100ml.

Solvent mixture

Chloroform, n-Heptane and methanol (all GR grade) were mixed in proportion of 49:49:2, respectively, and the mixture was designated as CHM.

Procedure

0.5 ml of plasma sample was taken in a 16×125 mm screw cap test tube. Then 0.1ml of 0.7 N HCl was added to the plasma sample. The mixture was shaken on a vortex test tube mixer. Following this, 2 ml of copper reagent and 6 ml of the solvent mixture added. The contents were shaken for 30 minutes on shaker at 240 rpm and then centrifuged for 10 minutes at 4°C at 3000 rpm in a refrigerated centrifuge. Solvent layer 3.5 ml was separated to an acid washed test tube containing 0.1 ml of the copper reagent. The contents were mixed well, then the colour intensity was measured within 1 hr at 440 nm using spectrometer against blank prepared in the same manner and using 0.5 ml double distilled water in place of plasma. The content of NEFA can be calculated from the standard curve.

Preparation of Standard Curve

The standard curve was prepared with palmitic acid as specified by Koop and Klomp (1977) as under 0.2 M solution of palmitic acid (5.12 g/100 ml) was prepared in solvent mixture as described above. One ml of this stock solution was diluted to 100ml with solvent mixture giving the final concentration of 2,000 µmol/lit 0.1 ,0.2, and 0.8ml aliquots of this solution having a concentration of 0.2,0.4,0.8,and 1.6 µmol of palmitic acid were taken and the colour was developed in the similar manner as given in procedure described

above. The blank was prepared simultaneously without palmitic acid. The concentration of palmitic acid was plotted against absorbance recorded at 440 nm. The values were expressed as µmol NEFA/liter of plasma.

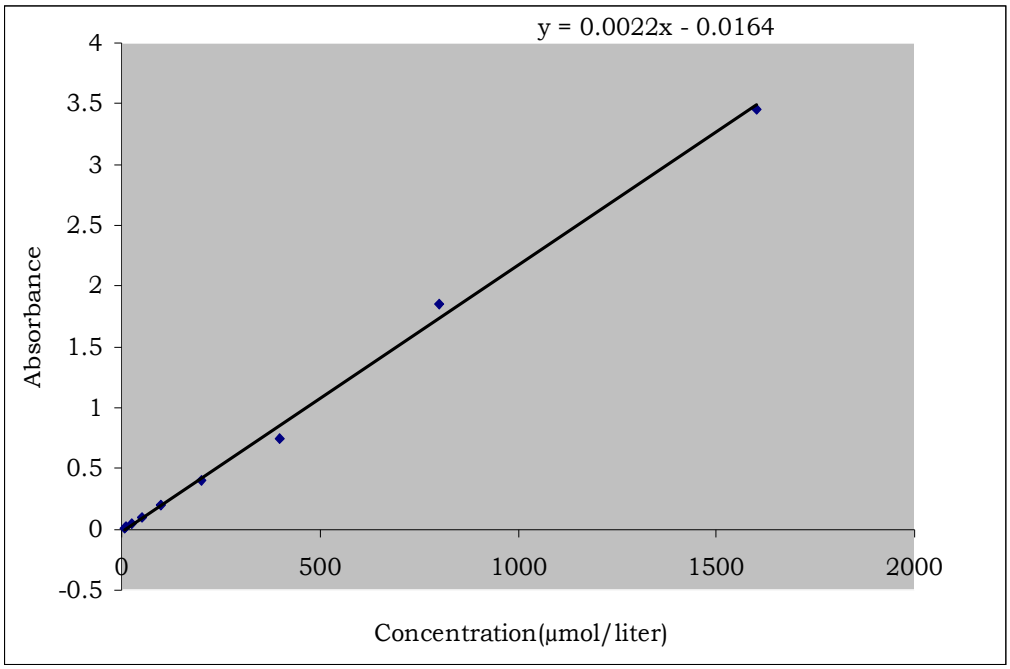


Figure 3.1 Standard Curve for NEFA estimation

3.2.4.6 Estimation of Trace Minerals (Cu, Fe, Mn and Zn)

One ml of plasma sample was digested with 5 ml of triacid mixture (HNO_3: $HClO_4$: H_2SO_4:: 3 : 2 : 1) in 60 ml test tubes. Tubes were heated till the contents were clear and perchloric acid fumes ceased to come out. The volume was made to 5 ml with double distilled water.

Assay: Standard solutions of copper, zinc, iron and manganese were prepared. The estimation of minerals was done on Philips Scientific model PU 9100 X Atomic Absorption Spectrophotometer (AAS) using acetylene as fuel and air as oxidant. Specific hollow cathode lamps

were used for the determination of various elements. The instrumental conditions described in AAS (1988) manual were followed.

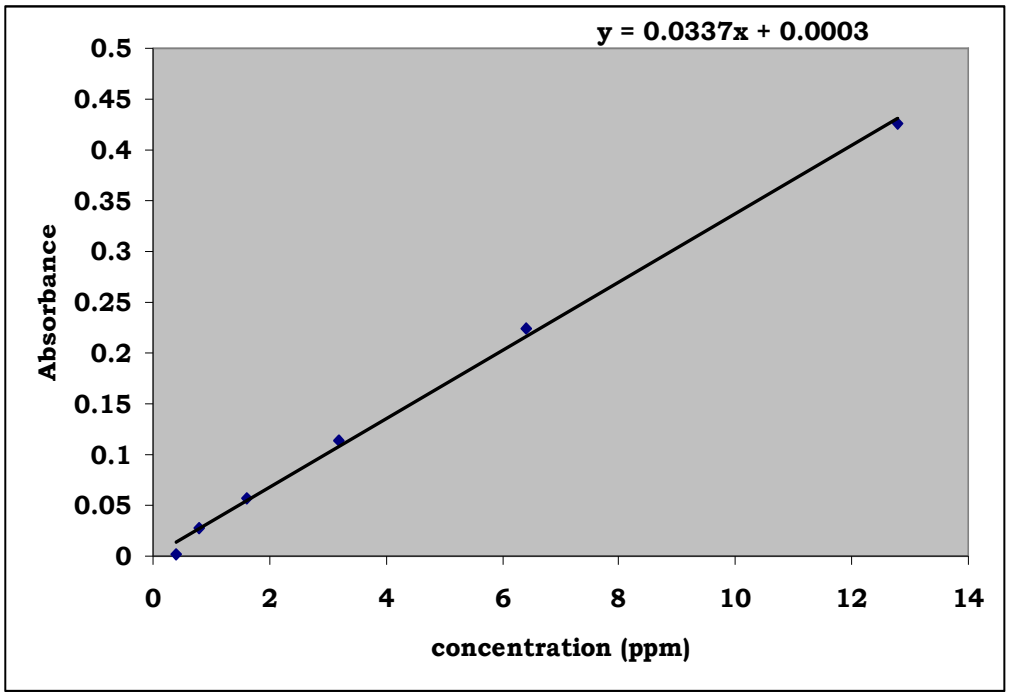


Figure 3.2 Standard Curve for Copper estimation

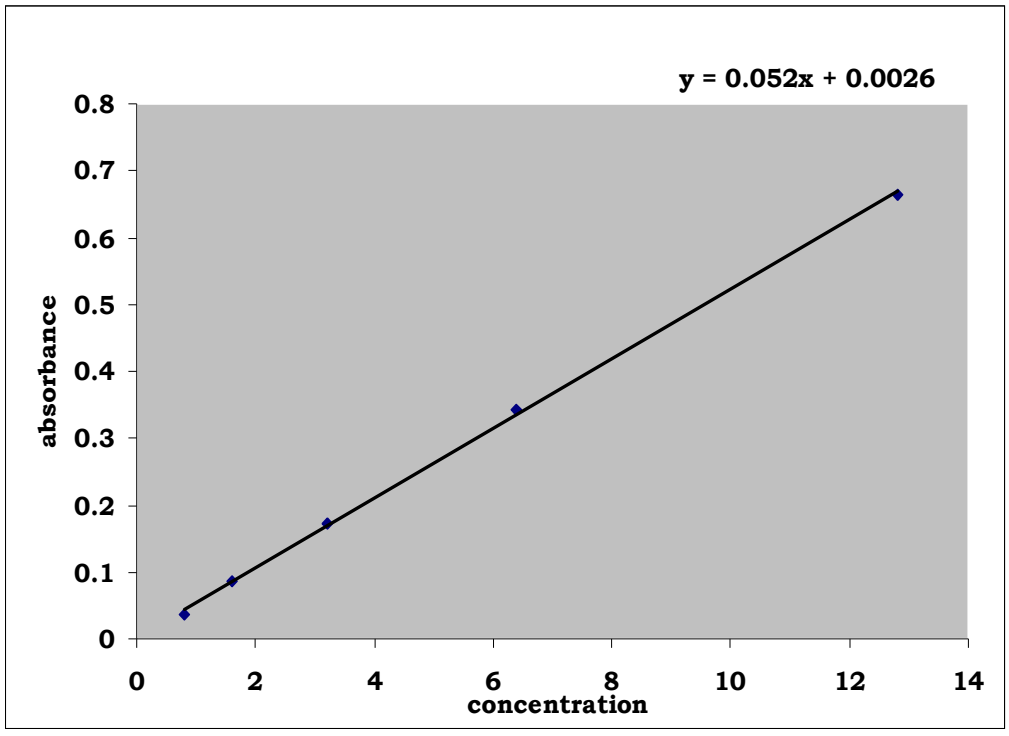


Figure 3.3 Standard Curve for Iron estimation

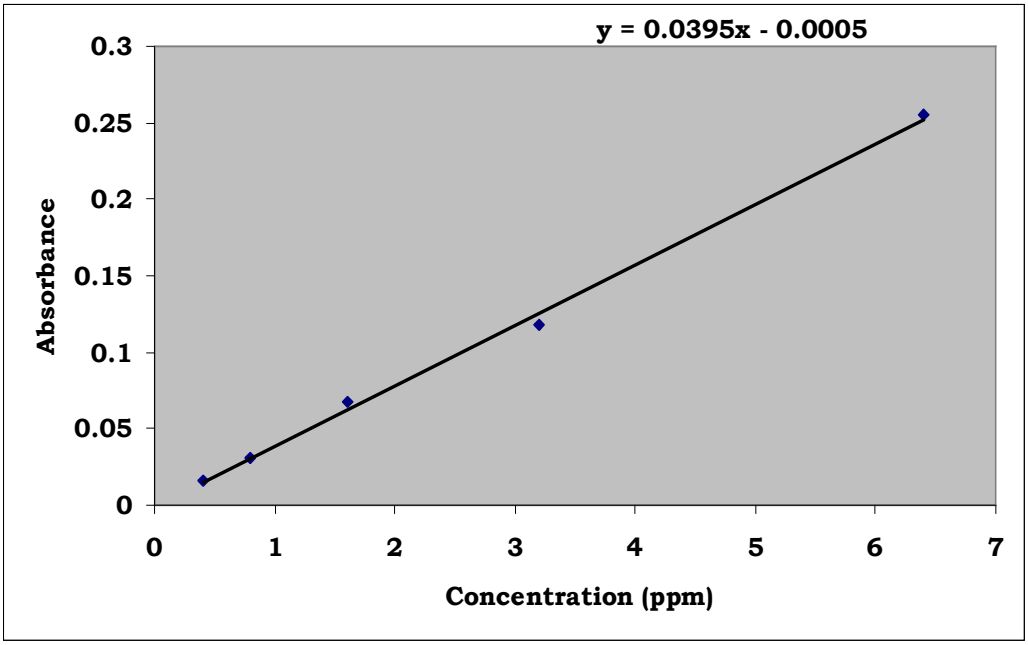


Figure 3.4 Standard Curve for Manganese estimation

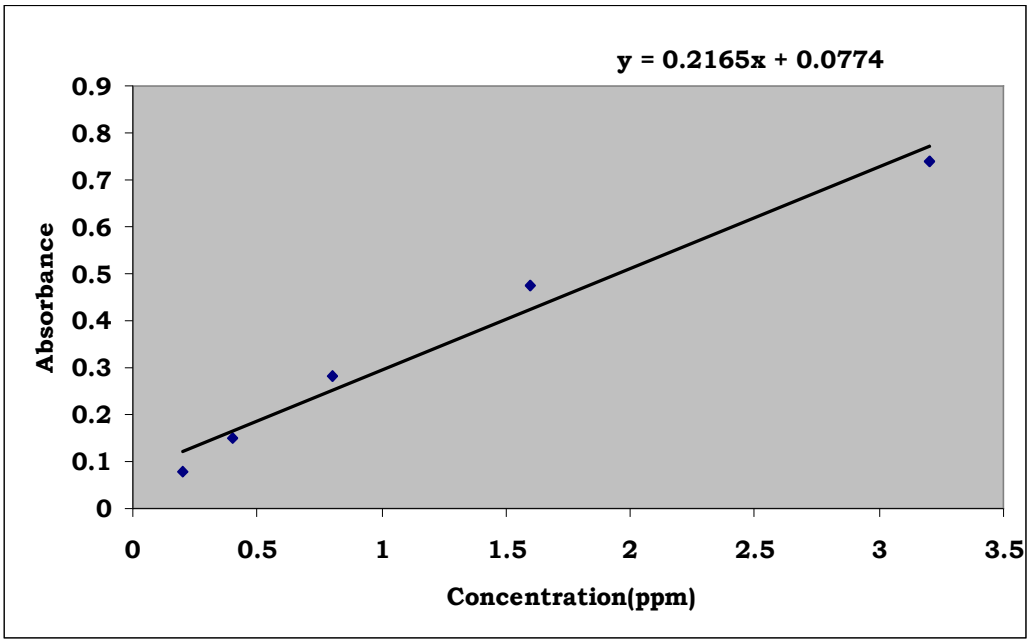


Figure 3.5 Standard Curve for Zinc estimation

3.2.4.7 Estimation of plasma Immunoglobulin G

Plasma IgG concentration was estimated by ELISA using Koma biotech, Korea kit.

Buffer Preparation

1. Coating Buffer: 50 mM carbonate-biocarbonate buffer, pH 9.6.

2. Washing Solution: 1 ml of 50% Tween 20 was added to 1 liter PBS and mixed.

3. Sample/Standard/Antibody Diluting Solution: Washing solution was used to dilute the plasma samples, standard and detection antibody.

4. Blocking solution: 10 mM phosphate, 0.14 M Nacl, 1% BSA, pH 7.4

5. Color Reaction Mixture: Mix 1 volume of TBM solution and 2 volume of substrate solution (H_2O_2) prior to use.

Sandwich ELISA Protocol

Coating

Capture antibody was diluted with coating buffer in 1: 100 in ratio and each well was coated with 100 µl of diluted capture antibody. Plates were incubated for over night at 40 ^{0} C. After incubation solution of each well was removed and each well was washed 3 times with washing solution.

Blocking

200 µl of blocking solution was added to each well and was incubated for 1 hour at room temperature. After incubation solution of each well was removed and each well was washed 3 times with washing solution.

Reacting Standards and Samples

Standard was diluted by diluting solution in 1: 50,000 ratios. Diluted standard was further diluted serially at 1:2 levels. Samples are diluted at 1:40 using diluting solution. 100 µl of standard or sample was transfer to assigned wells and Incubated for 1 hour at room

temperature. After incubation standards and samples of each well were removed and each well was washed 3 times with washing solution.

Detection antibody

Dilute the detection antibody in antibody diluting solution at a level of 1:25000. Add 100 µl of diluted antibody in each well and incubate for 1 hour at room temperature. Remove the solution and wash the well 3 times with washing solution.

Colour reaction and reading

Add 150 µl of color reaction mixture (50 µl TMB solution and 100 µl substrate solution) to each well. Mix well by shaking slightly.

Calculation of Results

Average the each duplicate reading from each standard control and sample. Subtract the zero reading from each average value above. Create the standard curve and find out the standard cure equation to calculate the IgG concentration.

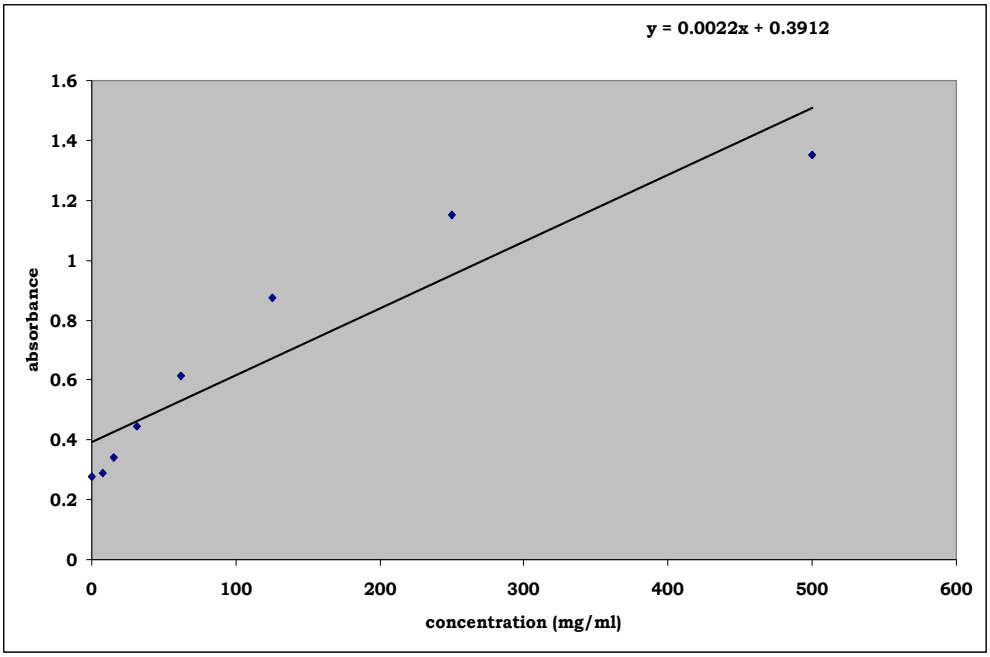


Figure 3.6 Standard Curve for Immunoglobulin G estimation

3.2.5 Postpartum Disorders

Cows were observed for the incidence of following postpartum disorders.

3.2.5.1 Retained Fetal Membranes

Bovine retained fetal membranes (RFM) have been defined as failure to expel fetal membranes within 8 to 12 hours postpartum.

3.2.5.2 Metritis

Metritis is the inflammation of the uterus. Metritis is mostly septic following parturition and is observed usually within 1 to 10 days after parturition. It is usually associated with removal of retention of fetal membranes, prolonged dystocia and abortion.

3.2.5.3 Endometritis

Endometritis is the inflammation of endometrial lining, the inner layer of the uterus without systemic signs, which is associated with chronic postpartum infection of the uterus with pathogenic bacteria.

3.2.5.4 Clinical Mastitis

Cows under experiment were examined for swelling and inflammation of teats at milking. Abnormal quarter were recorded and reviewed by expert veterinarian available at the institute.

3.2.6 Reproduction Performance

Reproductive performance of cows in different treatment groups was assessed for following parameters

a. Average number of days to postpartum oestrus

b. Average number of days to first service

c. Conception rate

d. Average Service period

e. Number of services/conception

3.2.7 Economics of Production

Production economics was determined by taking in to account expenditure on polyherbal supplementation, medicine for treatment of diseases and extra feeding due to prolonged service period and return from sale of milk. Following parameters were studied.

3.2.7.1 Cost of per Kg milk production

Cost of per liter milk production was calculated by dividing average (feeding+ supplementation+ treatment +extra feeding cost due to prolonged service period) daily expenditure from average daily milk yield as follows:

$$\textit{Cost Per Kg milk production (Rs./c/d)} = \frac{\textit{Average daily expenditure (Rs.)}}{\textit{Average daily milk production (Kg)}}$$

3.2.7.2 Gross returns

Gross returns were obtained by multiplying milk yield of an individual animal with respective prevailing prices in the study area, i.e.

Gross Return (Rs./cow/day) = Quantity of milk X Market price of milk

The market price of milk was considered as the rate prevailing at dairy cooperative near the experimental site and it was Rs. 16/kg.

3.2.7.3 Feeding Cost Saved due to Reduction of Service Period (Rs./cow/day)

Cost saved per day reduction of service period was calculated based on feeding cost per day. Total feeding cost of additional service period was divided to the service period of T_3 treatment group, in which service period was observed minimum.

Total feeding cost of extra long SP

Cost saved/ day reduction of S P= ----------

SP of T_3 treatment group

3.2.7.4 Income over Expenditure (Rs./cow/day)

Income from sale of milk over expenditure was calculated by subtracting average daily gross return from average daily (feeding+ supplementation+ treatment+ extra feeding cost due to prolonged service period) expenditure.

Average daily income = Average daily gross return - Average daily expenditure

3.3 Statistical Analysis

Data was subjected to analysis of variance using following statistical models.

Experiment 1: $Y_{ijkl} = \mu + S_i + P_j + M_k + E_{ijkl}$

Where,

Y_{ijkl} = Performance of cow in i^{th} season of p^{th} season of K^{th} period of mastitis

μ= population mean

S_i = Effect of i^{th} season (1, 2, 3,4)

P_j= Effect of j^{th} parity (1=1, 2 and 3=2, ≥4=3)

M_k = Effect of K^{th} time of mastitis (Before average service period=1, after average service period =2)

E_{ijk}= Random error

Experiment 2: $Y_{ij} = \mu + T_i + P_j + E_{ijk}$

Where,

Y_{ij} = Performance of cow in i^{th} treatment during j^{th} period

μ= population mean

T_i = Effect of i^{th} treatment (C, T_1, T_2 and T_3)

P_j =Effect of j^{th} period

E_{ijk}= Random error

Chapter Four

Results and Discussion

Chapter Four

RESULTS AND DISCUSSION

4.1 EFFECT OF TIME OF OCCURRENCE OF CLINICAL MASTITIS ON PRODUCTION AND REPRODUCTION PERFORMANE

Data pertaining to the production and reproduction performance of cows which got affected with clinical mastitis either before or after 'average service period' of herd (134 days of lactation) are presented in the table 4.1. These results reveal that; out of the total number of animals affected from clinical mastitis, a majority (61.20%) was either in immediate postpartum period or in early lactation. On the other hand, only 38.20% of the cows suffered from clinical mastitis during rest of the lactation. Total lactation yield and lactation length was significantly ($p \leq 0.05$) lower in cows showing onset of clinical mastitis before average service period. Although, no significant difference was observed in 305 days milk yield yet it was numerically higher in cow suffering from clinical mastitis before average service period of the herd. This might be due to the fact that a majority of the cows which suffered from clinical mastitis during early lactation before average service period of the herd recovered before the peak milk yield period of lactation. Average service period was significantly higher for the cows showing onset of clinical mastitis after average service period. This might be attributed to the fact that cows normally exhibit postpartum heat after 70-90 days of calving and the cows showing onset of clinical mastitis before average service period would have recovered before this period while cows which showed apparent signs of mastitis after average service period would be suffering from sub-clinical mastitis during the breeding period of the cows. As per Schrick *et al.* (2001) both the clinical and sub-clinical mastitis have similar negative effect on reproductive performance of the cows. Further, sub-clinical mastitis followed by clinical mastitis is more detrimental to the production as well as reproduction performance of the animals than clinical mastitis alone. Therefore, it can be inferred from these findings

that immediate post partum period and early lactation period are more critical from the udder health point of view and warrant special attention.

Table 4.1
Effect of clinical mastitis on performance of Karan-Fries cows

Group	N	Service period (days)	Lactation yield (Kg)	305 days yield (Kg)	Lactation length (days)
Before average service period (<134days)	336	155.79[a] ±7.64	4190.87 [a] ±90.96	3923.73 ±133.00	348.95 [a] ±5.87
After average service period (≥134 days)	213	210.94[b] ±9.32	4633.50 [b] ±110.91	3811.32 ±162.17	395.45 [b] ±7.16

Means bearing different superscripts within a column differ significantly (p≤0.05)

4.2 PRODUCTION PERFORMANCE

4.2.1 Daily Milk Yield

Daily milk yield data presented in Table 4.2 reveals that among the treatment groups, milk yield of cows in T_3 (polyherbal supplementation @ 200 mg/Kg BW) and T_2 (polyherbal supplementation @ 150 mg/Kg BW) during supplementation period, residual period as well as post-residual period was significantly (p≤0.05) higher than control and T_1(polyherbal supplementation @ 100 mg/Kg BW). However, there was no significant difference between T_3 and T_2 and between control and T_1. In comparison to control, milk yield in T_3 was 12.24, 15.01 and 10.50% higher during supplementation, residual and post-residual period, respectively. Whereas milk yield in T_2 during supplementation, residual and post-residual period, respectively, was 8.67, 11.46 and 10.50% higher than control. However, daily milk yield for 180 days of post calving period, in T_3 and T_2, respectively, was 12.72 and11.35% higher than control. Over the periods, milk yield from supplementation to residual

period, in control, T_1, T_2 and T_3 decreased by 5.56, 4.40, 2.58 and 2.48%, respectively, however, this reduction was significant ($p \leq 0.05$) only in control and T_1. From residual to post residual period, daily milk yield dropped significantly ($p \leq 0.05$) in all groups. This reduction was 14.37, 14.15, 11.46 and 18.64% in control, T_1, T_2 and T_3, respectively. Graphical representation of milk production data reveals that, In comparison to control and T_1, daily milk yield, even after suspension of supplementation remained higher in T_2 and T_3. Therefore it is evident from the results that polyherbal supplementation not only improved milk production but also sustained it at higher levels for a longer period even though there was a near consistent decline in milk yield during post supplementation period in all the treatment groups being the descending phase of lactation. Anjaria and Gupta (1967), Arora *et al.* (1983) Ramesh *et al.* (2000), Mishra *et al.* (2008) and Tanwar *et al.* (2008) with Shatavari based herbal preparation, also found similar results. This improvement in milk production can be attributed to better udder health and hormonal changes towards galactopoiesis. Udder health, as indicated by low incidence of sub-clinical mastitis (Table 4.24), low somatic cell count (Koeldeweij *et al.*,1999) (Table 4.22), low standard plate count (Table 4.23) and no incidence of clinical mastitis (Horpet *et al.*,1998) (Table 4.25), in higher supplemented groups (T_2 and T_3) was better than control and T_1. Shatavari, one of the polyherbal ingredients, contains some active components, which stimulate the hypothalamus or pituitary gland, leading to release of higher levels of prolactin hormone (Sabnis *et al.*, 1966; Ghosh *et al.*, 1987) thereby increasing the milk production. While estrogenic effect of shatavari on mammary glands, stimulates alveolar secretary epithelial cell division and proliferation (Sabnis *et al.*, 1966; Pandey *et al.*, 2005) which helps in sustenance of increased milk production.

4.2.2 Fat Percentage

Fat percentage data (Table 4.3) shows that during supplementation and residual period, fat% was significantly ($p \leq 0.05$) higher in T_2, followed by T_1, control and T_3. But during supplementation period, no significant difference was observed between control, T_1 and T_3; however, during residual period there was significant ($p \leq 0.05$) difference among control, T_1 and T_3. During post-residual period however, the fat% was higher ($p \leq 0.05$) in T_1, followed by T_2, control and T_3. Over the periods within the groups, except T_2, fat% in all groups increased significantly ($p \leq 0.05$), while, in T_2 it did not vary significantly (≤ 0.05). Fat % in control, T_1, T_2 and T_3 increased by 2.66, 5.97, 0.00 and 1.79%, respectively, with corresponding decrease of 19.01, 17.93, 13.75 and 20.67% milk production.

Table 4.2 Means (±SE) of daily milk yield (kg) in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/Kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	16.36^{a} ±0.33	19.06^{aC} ±0.17	16.58^{a}±0.44	18.85^{aB} ±0.18	17.54^{a} ±0.33	20.87^{bB} ±0.16	18.58^{b} ±0.41	21.72^{bB} ±0.22
2		19.51^{a} ±0.27		19.21^{a} ±0.32		21.49^{b} ±0.26		21.52^{b} ±0.40	
3		20.18^{a} ±0.32		19.99^{a} ±0.31		21.80^{b} ±0.29		22.60^{b} ±0.45	
4		19.29^{a} ±0.34		18.85^{a} ±0.34		21.53^{b} ±0.26		23.12^{c} ±0.40	
5	Residual (3-4 month)	18.64^{a} ±0.37	18.00^{aB} ±0.20	18.34^{a} ±0.36	18.02^{aA} ±0.16	21.17^{b} ±0.27	20.33^{bB} ±0.12	23.83^{c} ±1.38	21.18^{bB} ±0.38
6		18.53^{a} ±0.40		18.34^{a} ±0.32		20.62^{b} ±0.25		21.42^{b} ±0.32	
7		17.63^{a} ±0.37		17.86^{a} ±0.28		19.82^{b} ±0.20		20.25^{b} ±0.33	
8		17.21^{a} ±0.42		17.53^{a} ±0.28		19.71^{b} ±0.18		19.23^{b} ±0.27	
9	Post-residual (5-6 month)	16.82^{a} ±0.42	15.42^{aA} ±0.23	16.10^{a} ±0.34	15.47^{aA} ±0.13	19.50^{b} ±0.17	18.00^{bA} ±0.10	18.57^{b} ±0.28	17.23^{bA} ±0.14
10		15.96^{a} ±0.47		16.04^{a} ±0.23		18.60^{b} ±0.18		17.75^{b} ±0.28	
11		14.95^{a} ±0.47		15.18^{a} ±0.22		17.40^{b} ±0.16		16.79^{b} ±0.27	
12		13.94^{a} ±0.46		14.58^{a} ±0.19		16.50^{b} ±0.20		15.82^{b} ±0.25	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Table 4.3 Means (±SE) of fat % in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	4.33^{a}±0.02	4.39^{aA} ±0.01	4.50^{b} ±0.04	4.41^{aA} ±0.01	4.51^{b} ±0.03	4.60^{bA} ±0.01	4.36^{a} ±0.03	4.38^{aB} ±0.01
2		4.34^{a} ±0.02		4.41^{a} ±0.03		4.59^{b} ±0.02		4.34^{a} ±0.02	
3		4.41^{a} ±0.02		4.38^{a} ±0.02		4.67^{b} ±0.02		4.37^{a} ±0.02	
4		4.45^{a} ±0.02		4.43^{a} ±0.01		4.59^{b} ±0.03		4.45^{a} ±0.03	
5	Residual (3-4 month)	4.45^{a} ±0.02	4.40^{bA} ±0.01	4.46^{a} ±0.01	4.48^{cB} ±0.01	4.53^{b} ±0.03	4.56^{dA} ±0.01	4.47^{a} ±0.03	4.30^{aA} ±0.01
6		4.37^{b} ±0.02		4.44^{b} ±0.02		4.53^{c} ±0.03		4.29^{a} ±0.02	
7		4.37^{b} ±0.02		4.49^{c} ±0.02		4.54^{c} ±0.02		4.19^{a} ±0.02	
8		4.41^{b} ±0.02		4.57^{bc} ±0.02		4.63^{c} ±0.02		4.25^{a} ±0.02	
9	Post-residual (5-6 month)	4.46^{b} ±0.01	4.51^{aB} ±0.01	4.66^{c} ±0.02	4.69^{cB} ±0.01	4.66^{c} ±0.02	4.60^{bA} ±0.01	4.32^{a} ±0.03	4.46^{aC} ±0.01
10		4.51^{b} ±0.02		4.69^{d} ±0.02		4.59^{c} ±0.02		4.42^{a} ±0.02	
11		4.49^{a} ±0.03		4.69^{b} ±0.03		4.55^{a} ±0.02		4.53^{a} ±0.02	
12		4.57^{a} ±0.03		4.71^{b} ±0.03		4.58^{a} ±0.02		4.59^{a} ±0.02	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

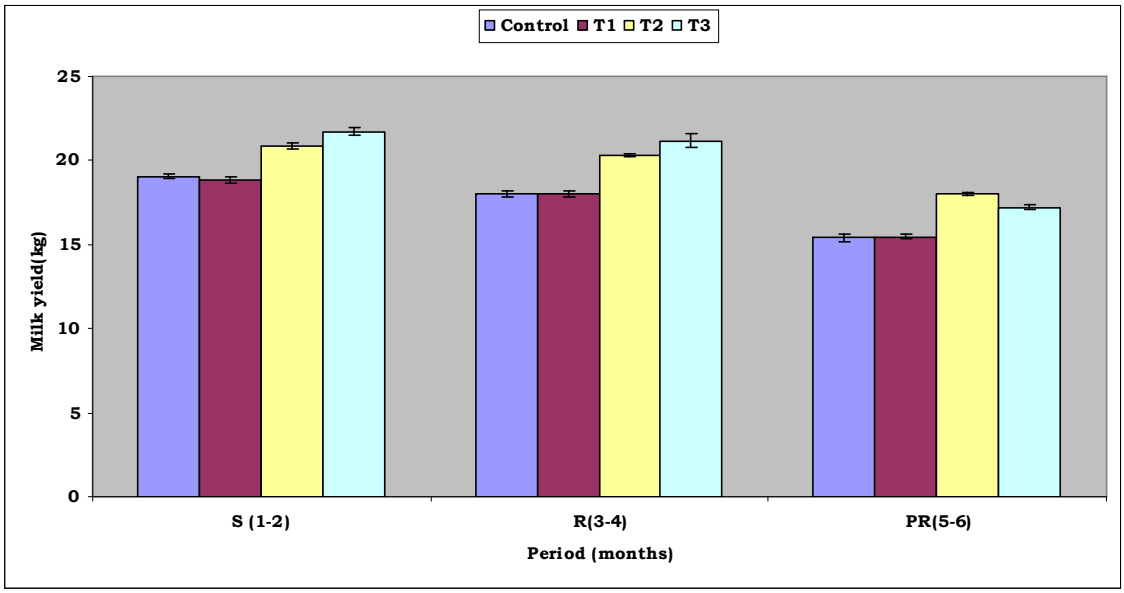


Figure 4.1
Average milk yield (Kg/day/cow) of treatment groups during different periods

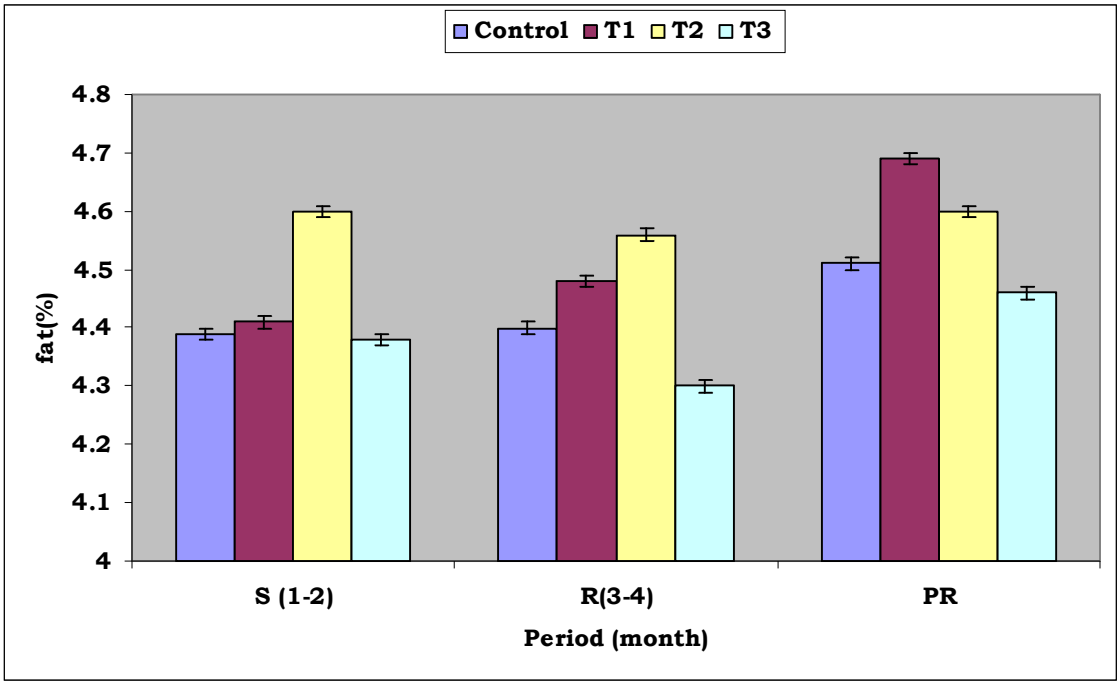


Figure 4.1
Average fat % of different treatment groups during different periods

These results suggest that in addition to galactopoietic effect, polyherbal supplementation also improved fat %, but as fat% is inversely proportional to milk production, therefore at higher supplementation rate the rate of increase in fat content of milk was apparently offset by galactopoietic effect. The results were in conformity with the findings of Berhane (2000) and Berhane and Singh (2002) who observed no significant improvement in milk fat percent on supplementing Shatavari in freshly calved crossbred cows. However, Santosh (2009) reported improvement in fat % of cows supplemented with Shatavari during pre and post partum period.

4.2.3 Fat Yield

Fat yield data (Table 4.5) indicates that during all periods, daily fat yield in T_2 and T_3 was significantly higher ($p \leq 0.05$) than control and T_1. Although, during supplementation and residual period, no significant difference in fat yield was observed between control and T_1 and between T_2 and T_3, but during post residual period fat yield in T_3 was significantly higher than T_2. Higher fat yield in T_2 and T_3 can be attributed to higher milk yield (Table 4.2) with insignificant depression in fat % (Table 4.4).

4.2.4 Fat Corrected Milk Yield (FCMY)

Fat corrected milk yield data (Table 4.3) indicated that daily fat corrected milk yield in T_2 and T_3, for entire experimental period was significantly higher ($p \leq 0.05$) than control and T_1. Although, during supplementation and residual period no significant difference between FCMY was observed between control and T_1 and between T_2 and T_3, but during post residual period FCMY in T_3 was significantly higher than T_2. This higher FCMY can be ascribed to high milk yield without a decrease in fat% in higher supplemented group.

Table 4.4 Means (±SE) of fat yield (kg) in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	0.71^{a}±0.02	0.83^{aC} ±0.01	0.75^{ab} ±0.02	0.83^{aB} ±0.01	0.79^{b} ±0.02	0.96^{bB} ±0.01	0.81^{ab} ±0.02	0.95^{bC} ±0.01
2		0.85^{a} ±0.01		0.84^{a} ±0.01		0.99^{b} ±0.01		0.94^{b} ±0.02	
3		0.89^{a} ±0.01		0.87^{a} ±0.01		1.02^{b} ±0.02		0.99^{b} ±0.02	
4		0.85^{a} ±0.01		0.82^{a} ±0.01		0.99^{b} ±0.01		1.03^{b} ±0.02	
5	Residual (3-4 month)	0.82^{a} ±0.01	0.79^{aB} ±0.01	0.81^{a} ±0.02	0.81^{aB} ±0.01	0.96^{b} ±0.01	0.93^{bB} ±0.01	1.06^{b} ±0.06	0.91^{bB} ±0.02
6		0.81^{a} ±0.02		0.81^{a} ±0.01		0.94^{b} ±0.01		0.92^{b} ±0.02	
7		0.77^{a} ±0.02		0.80^{a} ±0.01		0.90^{b} ±0.01		0.85^{b} ±0.02	
8		0.76^{a} ±0.02		0.80^{ab} ±0.01		0.91^{c} ±0.01		0.82^{b} ±0.01	
9	Post-residual (5-6 month)	0.75^{a} ±0.02	0.69^{aA} ±0.01	0.75^{a} ±0.02	0.72^{aA} ±0.01	0.91^{b} ±0.01	0.83^{cA} ±0.01	0.81^{c} ±0.01	0.77^{bA} ±0.01
10		0.71^{a} ±0.02		0.75^{ab} ±0.01		0.85^{c} ±0.01		0.79^{b} ±0.01	
11		0.67^{a} ±0.02		0.71^{a} ±0.01		0.79^{b} ±0.01		0.76^{b} ±0.01	
12		0.64^{a} ±0.02		0.68^{ab} ±0.01		0.76^{b} ±0.01		0.73^{b} ±0.01	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Table 4.5 Means (±SE) of fat corrected milk yield (kg) in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	$17.19^{a}\pm0.36$	$20.15^{aC}\pm0.18$	$17.83^{ab}\pm0.50$	$19.94^{aB}\pm0.18$	$18.89^{bc}\pm0.37$	$22.77^{bB}\pm0.18$	$19.65^{c}\pm0.48$	$23.01^{bB}\pm0.25$
2		$20.50^{a}\pm0.28$		$20.32^{a}\pm0.34$		$23.39^{b}\pm0.30$		$22.65^{b}\pm0.46$	
3		$21.39^{a}\pm0.33$		$21.03^{a}\pm0.28$		$24.02^{b}\pm0.35$		$23.87^{b}\pm0.50$	
4		$20.52^{a}\pm0.35$		$19.87^{a}\pm0.33$		$23.48^{b}\pm0.31$		$24.74^{b}\pm0.48$	
5	Residual (3-4 month)	$19.79^{a}\pm0.36$	$19.04^{aB}\pm0.20$	$19.50^{a}\pm0.38$	$19.31^{aB}\pm0.17$	$22.85^{b}\pm0.31$	$22.05^{bB}\pm0.14$	$25.48^{b}\pm1.39$	$22.19^{bB}\pm0.39$
6		$19.52^{a}\pm0.41$		$19.55^{a}\pm0.35$		$22.28^{b}\pm0.29$		$22.41^{b}\pm0.37$	
7		$18.62^{a}\pm0.39$		$19.14^{a}\pm0.30$		$21.48^{b}\pm0.25$		$20.88^{b}\pm0.37$	
8		$18.26^{a}\pm0.45$		$19.03^{ab}\pm0.32$		$21.58^{c}\pm0.21$		$19.99^{b}\pm0.30$	
9	Post-residual (5-6 month)	$17.94^{a}\pm0.43$	$16.55^{aA}\pm0.24$	$17.66^{a}\pm0.36$	$17.02^{aA}\pm0.13$	$21.41^{c}\pm0.18$	$19.61^{bA}\pm0.11$	$19.50^{b}\pm0.32$	$18.44^{cA}\pm0.16$
10		$17.09^{a}\pm0.49$		$17.63^{a}\pm0.23$		$20.24^{c}\pm0.19$		$18.90^{b}\pm0.31$	
11		$16.02^{a}\pm0.48$		$16.69^{a}\pm0.22$		$18.87^{b}\pm0.19$		$18.15^{b}\pm0.31$	
12		$15.15^{a}\pm0.49$		$16.10^{ab}\pm0.22$		$17.93^{c}\pm0.22$		$17.22^{b}\pm0.28$	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

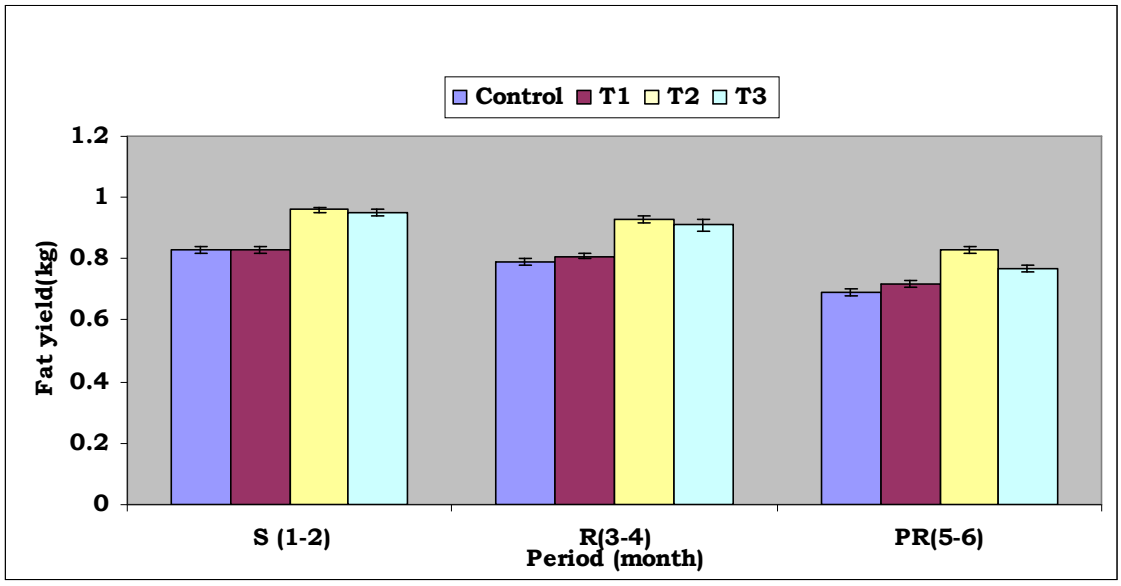


Figure 4.3
Average Fat yield (Kg/cow/day) of treatment groups during different periods

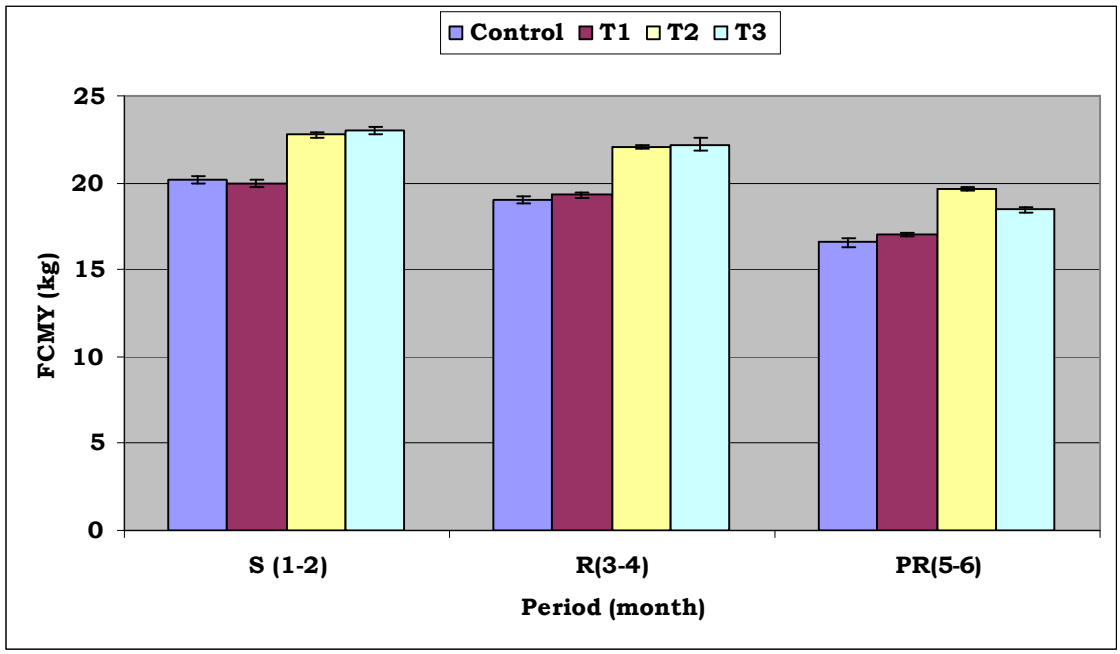


Figure 4.4
Average FCM yield (Kg/ cow /day) of the treatment groups during different periods

4.2.5 Protein Percentage

Data on Protein percentage presented in table 4.6 reveal that during supplementation and residual periods, protein % in control group was significantly ($p \leq 0.05$) higher than T_1, T_2 and T_3. However, during post-residual period, protein % in T_2 and T_3 was significantly ($p \leq 0.05$) higher than control and T_1. There appeared to be a decline in milk protein content of the treatment groups T_1, T_3 as well as control from the initial treatment period to the residual and post residual period. In treatment group T_2 the decline was not consistent. From supplementation to residual period, protein % in all groups reduced significantly ($p \leq 0.05$). Percent decrease was highest, 3.85% in T_3 followed by 3.76% in control, 1.62% in T_2 and 1.30% in T_1. From residual to post residual period, in control and T_1, protein % reduced significantly (($p \leq 0.05$), however, protein % in T_2 and T_3 showed increasing trends which was significant only in T_2. This variation in protein % in control, T_1, T_2 and T_3 was –3.58,-1.98, 1.63 and 0.60% and 3.85 %, respectively. Several factors like milk yield, seasonal variation and availability of rumen protected protein are reported to affect milk protein content. Milk protein % has also been shown to be negatively influenced by increase in milk production (Barman, 2004). In the present study also there were variable trends in protein content in milk among different treatment groups. Berhane (2000) and Berhane and Singh (2002) observed no change in milk protein percent in Shatavari supplemented crossbred cows.

4.2.6 Protein Yield

Protein yield data (Table 4.7) reveals that during supplementation and residual period, protein yield in T_3 was significantly ($p \leq 0.05$) higher than control, T_1 and T_2. However, only during supplementation period, significant difference existed among control, T_1 and T_2. During post-residual period, protein yield in T_2 and T_3 was significantly ($p \leq 0.05$) higher than control and T_1. But there was no significant difference

between control and T_1. This higher protein yield in T_2 and T_3 could be attributed to higher milk yield (Table 4.2) in relation to less protein% (Table 4.6) depression in these groups.

Table 4.6 Means (±SE) of protein% in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	3.34^{b} ±0.05	$3.19^{c\ C}$ ±0.02	3.11^{a} ±0.02	$3.07^{a\ C}$ ±0.01	3.07^{a} ±0.01	$3.07^{a\ B}$ ±0.01	3.15^{a} ±0.01	$3.12^{b\ B}$ ±0.01
2		3.19^{b} ±0.03		3.09^{a} ±0.01		3.09^{a} ±0.01		3.12^{a} ±0.01	
3		3.18^{b} ±0.03		3.06^{a} ±0.01		3.09^{a} ±0.01		3.11^{a} ±0.01	
4		3.09^{a} ±0.01		3.03^{a}±0.01		3.03^{a} ±0.01		3.12^{a} ±0.01	
5	Residual (3-4 month)	3.07^{b} ±0.01	$3.07^{b\ B}$ ±0.01	3.02^{a} ±0.01	$3.03^{a\ B}$ ±0.01	3.01^{a} ±0.01	$3.02^{\ a\ A}$ ±0.01	3.10^{a} ±0.01	$3.00^{a\ A}$ ±0.01
6		3.07^{c} ±0.01		3.01^{b} ±0.01		3.02^{b} ±0.01		2.99^{a} ±0.01	
7		3.09^{c} ±0.01		3.02^{b} ±0.01		3.02^{b} ±0.01		2.93^{a} ±0.01	
8		3.05^{c} ±0.01		3.02^{b} ±0.01		3.01^{b} ±0.01		2.98^{a} ±0.01	
9	Post-residual (5-6 month)	3.00^{a} ±0.01	$2.96^{a\ A}$ ±0.01	3.00^{a} ±0.01	$2.97^{a\ A}$ ±0.01	3.01^{a} ±0.01	$3.07^{c\ B}$ ±0.01	3.00^{a} ±0.01	$3.02^{\ b\ A}$ ±0.01
10		2.97^{a} ±0.01		2.96^{a} ±0.01		3.05^{b} ±0.01		2.96^{a} ±0.01	
11		2.95^{a} ±0.01		2.95^{a} ±0.01		3.10^{b} ±0.01		2.98^{a} ±0.01	
12		2.94^{a} ±0.01		2.96^{a} ±0.01		3.12^{b} ±0.01		3.12^{b} ±0.02	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Table 4.7 Means (±SE) of protein yield (kg) in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	0.55^{ab} ±0.01	0.61^{aC} ±0.01	0.52^{a} ±0.02	0.58^{bC} ±0.01	0.54^{ab} ±0.01	0.61^{cA} ±0.01	0.59^{b} ±0.01	0.68^{dC} ±0.01
2		0.63^{ab} ±0.01		0.59^{a} ±0.01		0.66^{b} ±0.01		0.67^{b} ±0.01	
3		0.64^{ab}±0.01		0.61^{a} ±0.01		0.67^{bc} ±0.01		0.70^{c} ±0.01	
4		0.60^{a} ±0.01		0.57^{a} ±0.01		0.65^{b} ±0.01		0.72^{c} ±0.01	
5	Residual (3-4 month)	0.57^{a} ±0.01	0.55^{aB} ±0.01	0.56^{a} ±0.01	0.55^{aB} ±0.01	0.64^{a} ±0.01	0.55^{aA} ±0.01	0.74^{b} ±0.01	0.64^{bB} ±0.01
6		0.57^{a} ±0.01		0.56^{a} ±0.01		0.62^{b} ±0.01		0.64^{b} ±0.01	
7		0.54^{a} ±0.01		0.54^{a} ±0.01		0.60^{b} ±0.01		0.59^{b} ±0.01	
8		0.53^{a} ±0.01		0.53^{a} ±0.01		0.59^{b} ±0.01		0.58^{b} ±0.01	
9	Post-residual (5-6 month)	0.50^{a} ±0.01	0.45^{aA} ±0.01	0.48^{a} ±0.01	0.46^{aA} ±0.01	0.59^{b} ±0.01	0.68^{cB} ±0.01	0.56^{b} ±0.01	0.52^{bA} ±0.01
10		0.47^{a} ±0.01		0.47^{a} ±0.01		0.57^{c} ±0.01		0.53^{b} ±0.01	
11		0.44^{a} ±0.01		0.45^{a} ±0.01		0.54^{c} ±0.01		0.50^{b} ±0.01	
12		0.41^{a} ±0.01		0.43^{a} ±0.01		0.51^{b} ±0.01		0.49^{b} ±0.01	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

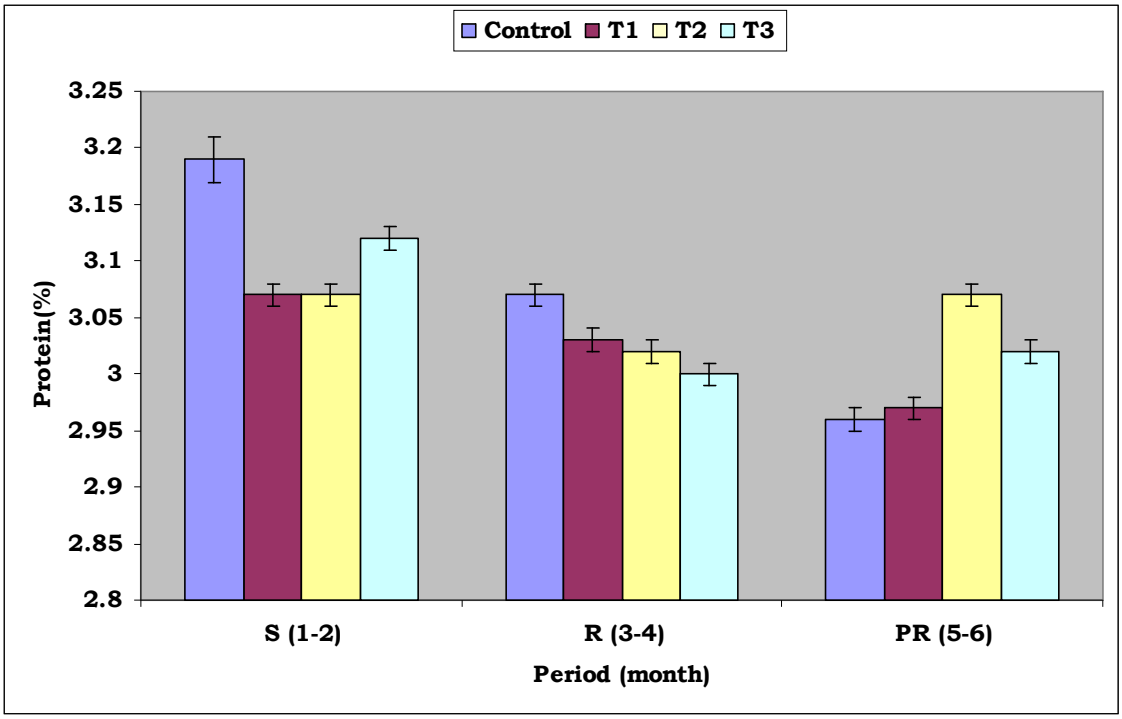


Figure 4.5
Average protein % of different treatment groups during different periods

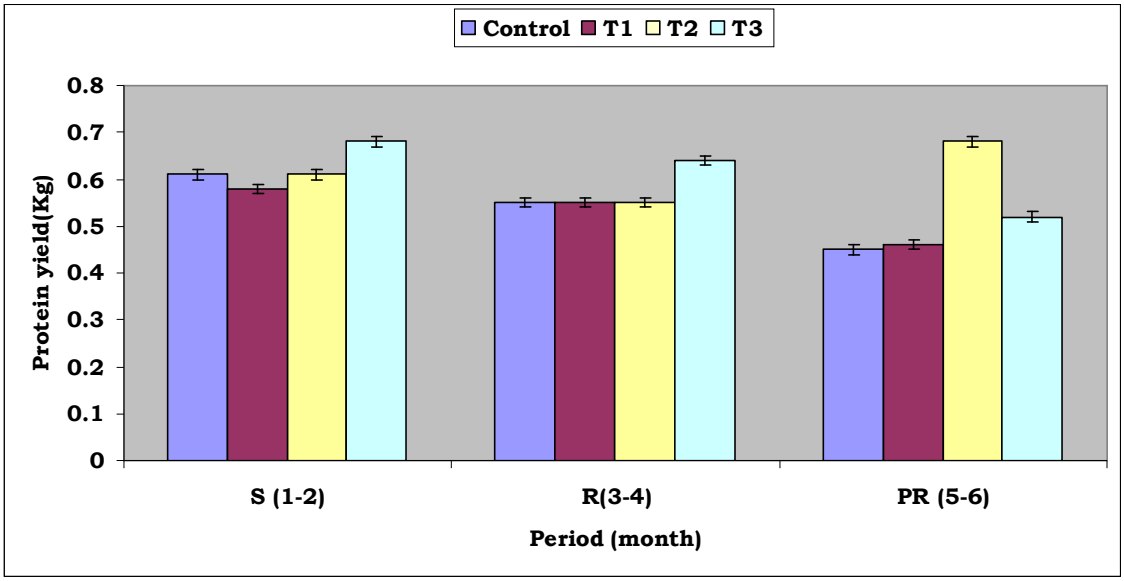


Figure 4.6
Average Protein yield (Kg/cow/day) of different treatment group during different periods

4.2.7 Lactose Percentage

Lactose percentage data (Table 4.8) revealed that during supplementation period, lactose % in T_1, T_2 and T_3 was significantly ($p \leq 0.05$) higher than control. But no significant difference was observed between T_1 and T_2. During residual period, only in T_3, lactose % was significantly ($p \leq 0.05$) higher than control. During post-residual period, lactose% in T_3 was non-significantly higher than control, followed by T_1 and T_2. From supplementation to residual period lactose% decreased significantly ($p \leq 0.05$) only in control and T_1, however no-significant depression was observed in T_2 and T_3. From residual to post-residual period, lactose% in none of the groups varied significantly ($p \leq 0.05$). Lactose % in highest supplemented group remained higher during entire observation period. These results suggest that polyherbal supplementation improved lactose % and its effect was sustained longer at higher dose rate. Mastitis is one of the important factor influencing milk yield and composition of the animals and has also been reported to cause significant reduction in milk lactose percentage (Harmon1994).Therefore, higher lactose% in higher supplemented groups can be associated with better udder health in these groups as indicated by low incidence of sub-clinical mastitis (Table 4.24), low somatic cell count (Table 4.22), low standard plate count (Table 4.23) and no incidence of clinical mastitis (Table 4.25).

4.2.8 Lactose Yield

Lactose yield data, presented in Table 4.9 reveal that during supplementation, residual and post-residual period, lactose yield in T_3 and T_2 was significantly($p \leq 0.05$) higher than T_1 and control. However, there was no significant difference between control and T_1. From supplementation to residual period, lactose yield decreased significantly ($p \leq 0.05$) in control and T_1. However, in T_2 and T_3 significant depression in lactose yield was observed only during post residual period. Higher lactose yield in T_2 and T_3 could be attributed to higher milk yield (Table 4.2) and lactose% (Table4.8)

Table 4.8 Means (±SE) of lactose % in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	4.65^{a} ±0.01	$4.69^{a\,B}$ ±0.12	$4.69^{a\,B}$±0.12	$4.71^{b\,B}$ ±0.15	4.67^{b} ±0.01	$4.71^{b\,A}$ ±0.07	4.76^{c} ±0.01	$4.75^{c\,A}$ ±0.10
2		4.68^{a} ±0.01		4.69^{a} ±0.01		4.71^{ab} ±0.01		4.74^{b} ±0.01	
3		4.71^{b} ±0.01		4.69^{a} ±0.01		4.73^{b} ±0.01		4.72^{ab} ±0.01	
4		4.73^{c} ±0.01		4.75^{bc} ±0.01		4.74^{b} ±0.01		4.77^{c} ±0.01	
5	Residual (3-4 month)	4.59^{a}±0.04	$4.67^{a\,A}$ ±0.23	4.74^{b} ±0.01	$4.67^{a\,A}$ ±0.10	4.73^{b} ±0.01	$4.67^{a\,A}$ ±0.10	4.79^{c} ±0.01	$4.75^{b\,A}$ ±0.10
6		4.68^{a} ±0.02		4.68^{a} ±0.01		4.70^{a} ±0.01		4.75^{b} ±0.01	
7		4.70^{ab} ±0.01		4.64^{a} ±0.01		4.65^{a} ±0.01		4.73^{b} ±0.01	
8		4.68^{a} ±0.01		4.62^{b} ±0.01		4.58^{a} ±0.01		4.71^{c} ±0.01	
9	Post-residual (5-6 month)	4.72^{d} ±0.01	$4.68^{c\,A}$ ±0.12	4.64^{b} ±0.01	$4.65^{b\,A}$ ±0.12	4.54^{a} ±0.01	$4.58^{a\,A}$ ±0.09	4.68^{c} ±0.01	$4.70^{c\,A}$ ±0.11
10		4.70^{c} ±0.01		4.66^{b} ±0.01		4.57^{a} ±0.01		4.69^{c} ±0.01	
11		4.67^{b} ±0.01		4.66^{b} ±0.01		4.59^{a} ±0.01		4.69^{b} ±0.01	
12		4.64^{b} ±0.01		4.63^{ab} ±0.02		4.60^{a} ±0.01		4.70^{c} ±0.01	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Table 4.9 Means (±SE) of lactose yield (kg) in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	0.75^{a} ±0.17	$0.89^{a\,B}$ ±0.01	0.78^{ab}±0.23	$0.89^{a\,B}$ ±0.01	0.82^{bc}±0.16	$0.98^{b\,C}$ ±0.01	0.88^{c}±0.20	$1.03^{c\,C}$ ±0.01
2		0.92^{a} ±0.18		0.90^{a}±0.19		1.01^{b}±0.15		1.02^{b}±0.23	
3		0.95^{a}±0.20		0.94^{a}±0.18		1.03^{b}±0.17		1.07^{b}±0.26	
4		0.91^{a}±0.22		0.90^{a}±0.20		1.02^{b}±0.15		1.10^{b}±0.24	
5	Residual (3-4 month)	0.87^{a}±0.22	$0.85^{a\,A}$ ±0.01	0.87^{a}±0.22	$0.84^{a\,A}$ ±0.01	1.00^{b}±0.15	$0.95^{b\,C}$ ±0.01	1.14^{c}±0.82	$1.01^{c\,C}$ ±0.01
6		0.87^{a}±0.24		0.86^{a}±0.20		0.97^{b}±0.14		1.02^{b}±0.20	
7		0.83^{a}±0.22		0.83^{a}±0.16		0.92^{b}±0.10		0.96^{b}±0.20	
8		0.81^{a}±0.25		0.81^{a}±0.16		0.90^{b}±0.09		0.91^{b}±0.16	
9	Post-residual (5-6 month)	0.79^{a}±0.24	$0.72^{a\,A}$ ±0.01	0.75^{a}±0.19	$0.72^{a\,A}$ ±0.01	0.89^{b}±0.08	$0.82^{b\,B}$ ±0.01	0.87^{b}±0.15	$0.81^{b\,B}$ ±0.01
10		0.75^{a}±0.27		0.75^{a}±0.14		0.85^{b}±0.10		0.83^{b}±0.15	
11		0.70^{a}±0.26		0.71^{a}±0.13		0.80^{b}±0.09		0.79^{b}±0.15	
12		0.65^{a}±0.26		0.68^{a}±0.12		0.76^{b}±0.11		0.74^{b}±0.14	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

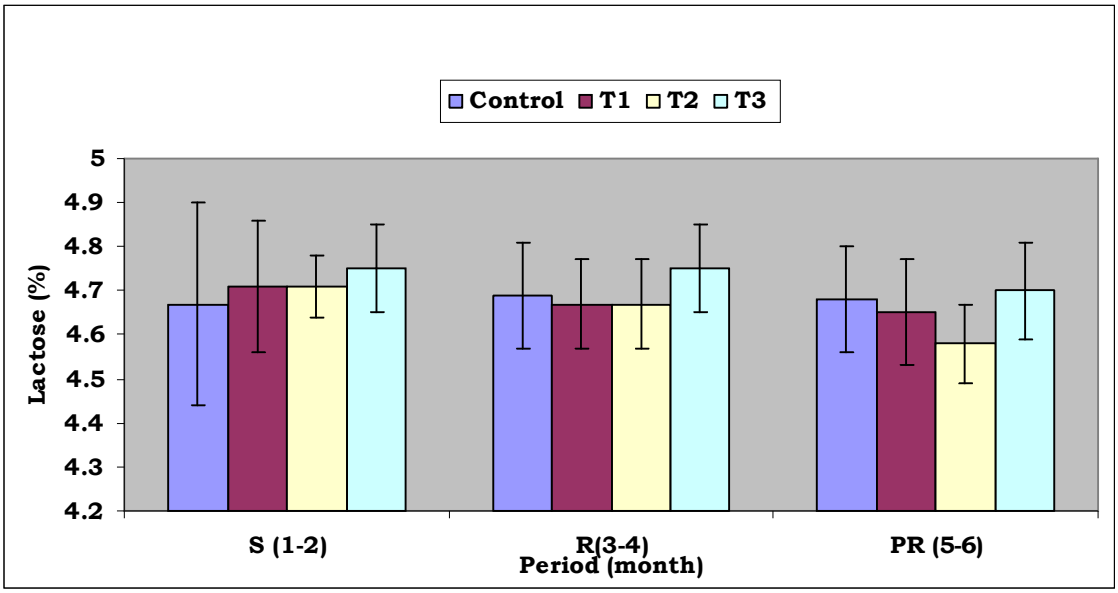


Figure 4.7
Average lactose % of different treatment groups during different periods

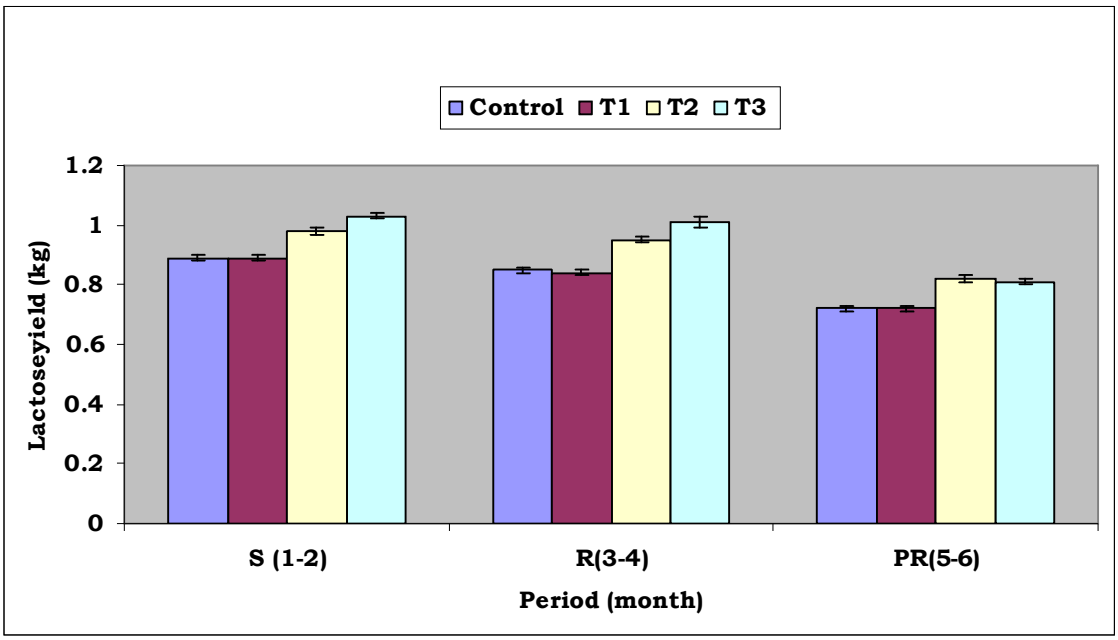


Figure 4.8
Average lactose yield (Kg/cow/day) of different treatment group during different periods

4.2.9 Solid Not Fat (SNF) Percentage

SNF % data presented in table 4.10 reveals that during supplementation period, SNF% in T_1, T_2 and T_3 was significantly ($p \leq 0.05$) higher than control, however, no significant difference was observed among T_1, T_2 and T_3. During residual period, SNF% in T_2 and T_3 was significantly ($p \leq 0.05$) higher than control and T_1, however, there was no significant difference between control and T_1 and between T_2 and T_3. During post residual period, among groups, no specific trends were observed. From supplementation to post-residual period, only control and T_1 showed significant ($p \leq 0.05$) increase in SNF%, however, over this period no significant variation was observed in T_1 and T_2. These results indicate that polyherbal supplementation had positive effect on SNF% and this positive effect continued even after discontinuation of polyherbal supplementation.

4.2.10 Solid Not Fat Yield

SNF yield data is presented in table 4.11. Results reveals that during supplementation, residual and post-residual period, SNF yield in T_3 and T_2 was significantly ($p \leq 0.05$) higher than T_1 and control. However, there was no significant difference between control and T_1. From supplementation to residual period, only in control and T_1, SNF yield decreased significantly ($p \leq 0.05$), however, in T_2 and T_3 significant depression in SNF yield was observed only in post residual period. This higher and persistent SNF yield in T_2 and T_3 could be attributed to higher milk yield (Table 4.2) and SNF% (Table 4.10) in these groups.

Table 4.10 Means (±SE) of SNF % in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	7.99^{a}±0.15	$8.46^{a\,A}$ ±0.04	8.75^{b} ±0.01	$8.70^{b\,A}$ ±0.01	8.68^{b} ±0.01	$8.73^{b\,A}$ ±0.01	8.71^{b} ±0.01	$8.73^{b\,A}$ ±0.01
2		8.45^{a} ±0.08		8.72^{b} ±0.01		8.70^{b} ±0.01		8.71^{b} ±0.01	
3		8.51^{a} ±0.07		8.68^{b} ±0.01		8.75^{b} ±0.01		8.73^{b} ±0.01	
4		8.72^{b} ±0.01		8.66^{a} ±0.01		8.77^{c} ±0.01		8.75^{c} ±0.01	
5	Residual (3-4 month)	8.71^{b} ±0.01	8.68^{aB} ±0.01	8.66^{a} ±0.01	$8.67^{a\,A}$ ±0.01	8.77^{c}±0.01	$8.74^{b\,A}$ ±0.01	8.76^{c} ±0.01	$8.72^{ab\,A}$ ±0.01
6		8.69^{b} ±0.01		8.67^{a} ±0.01		8.75^{c} ±0.01		8.73^{c} ±0.01	
7		8.67^{a} ±0.01		8.68^{a} ±0.01		8.72^{c} ±0.01		8.70^{b} ±0.01	
8		8.66^{a} ±0.01		8.69^{b} ±0.01		8.70^{b} ±0.01		8.70^{b} ±0.01	
9	Post-residual (5-6 month)	8.65^{a} ±0.01	8.66^{aB} ±0.01	8.72^{b} ±0.01	$8.75^{bc\,B}$ ±0.01	8.70^{c} ±0.01	$8.70^{a\,A}$ ±0.01	8.70^{ab} ±0.01	$8.72^{b\,A}$ ±0.01
10		8.65^{a} ±0.01		8.76^{c} ±0.01		8.70^{b} ±0.01		8.71^{b} ±0.01	
11		8.66^{a} ±0.01		8.77^{c} ±0.01		8.70^{b} ±0.01		8.72^{b} ±0.01	
12		8.66^{a} ±0.01		8.77^{c} ±0.01		8.71^{b} ±0.01		8.75^{c} ±0.01	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Table 4.11 Means (±SE) of SNF yield (kg) in different treatment groups

Fortnight	Period	Treatment							
		Control		T_1 (150 mg/kg BW)		T_2 (200 mg/kg BW)		T_3 (250 mg/kg BW)	
1	Supplementation (1-2month)	1.30^{a} ±0.04	1.61$^{a\ C}$ ±0.02	1.45^{b} ±0.04	1.64$^{a\ C}$ ±0.02	1.52^{bc} ±0.03	1.82$^{b\ B}$ ±0.01	1.62^{c} ±0.04	1.90$^{c\ B}$ ±0.02
2		1.65^{a} ±0.03		1.63^{a} ±0.03		1.87^{b} ±0.02		1.87^{b} ±0.04	
3		1.72^{a} ±0.03		1.73^{a} ±0.03		1.91^{b} ±0.03		1.97^{b} ±0.04	
4		1.68^{a} ±0.03		1.63^{a} ±0.03		1.89^{b} ±0.02		2.02^{c} ±0.04	
5	Residual (3-4 month)	1.62^{a} ±0.03	1.56$^{a\ B}$ ±0.02	1.59^{a} ±0.03	1.56$^{a\ B}$ ±0.01	1.86^{b} ±0.02	1.78$^{b\ B}$ ±0.01	2.09^{b} ±0.12	1.85$^{c\ B}$ ±0.03
6		1.61^{a} ±0.03		1.59^{a} ±0.03		1.80^{b} ±0.02		1.87^{b} ±0.03	
7		1.53^{a} ±0.03		1.55^{a} ±0.02		1.73^{b} ±0.02		1.76^{b} ±0.03	
8		1.49^{a} ±0.04		1.52^{a} ±0.03		1.72^{b} ±0.02		1.67^{b} ±0.02	
9	Post-residual (5-6 month)	1.45^{a} ±0.04	1.33$^{a\ A}$ ±0.02	1.40^{a} ±0.03	1.35$^{a\ A}$ ±0.01	1.70^{b} ±0.02	1.57$^{b\ A}$ ±0.01	1.62^{b} ±0.02	1.50$^{b\ A}$ ±0.01
10		1.38 a ±0.04		1.40^{a} ±0.02		1.62^{b} ±0.02		1.54^{b} ±0.02	
11		1.29 a ±0.04		1.33^{a} ±0.02		1.51^{b} ±0.02		1.46^{b} ±0.02	
12		1.20 a ±0.04		1.28^{a} ±0.02		1.44^{b} ±0.02		1.38^{b} ±0.02	

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05) A

Table: 4.12 Least squares analysis of variance for production parameters in different treatments at different periods

Parameter	MSS Period (2)	MSS Treatment (3)	MSS Period xTreatment (6)	MSS Error (6988)
Daily milk yield	8417.60*	3435.15*	105.72*	22.09
FCMY	8122.30*	4202.85*	101.09*	25.00
Fat%	12.20*	14.31*	2.22*	0.08
Protein%	7.43*	0.83*	1.45*	0.02
Lactose%	2.24*	1.70*	0.59*	0.01
SNF%	2.14*	6.30*	2.57*	0.08
Fat yield	12.69*	7.81*	0.19*	0.04
Protein yield	10.34*	3.20*	0.17*	0.02
Lactose yield	21.28*	7.93*	0.24*	0.05
SNF yield	61.36*	29.70*	0.82*	0.17

- Significant at 1% level of significance
- Figures in parenthesis indicate degree of freedom

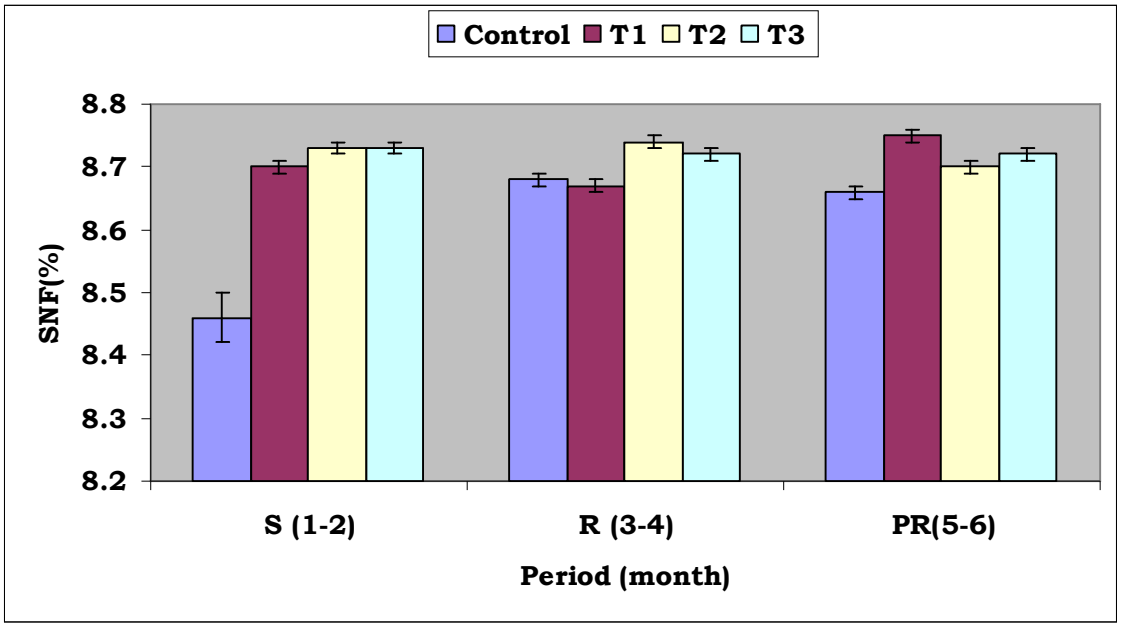


Figure 4.9
Average SNF % of different treatment groups during different periods

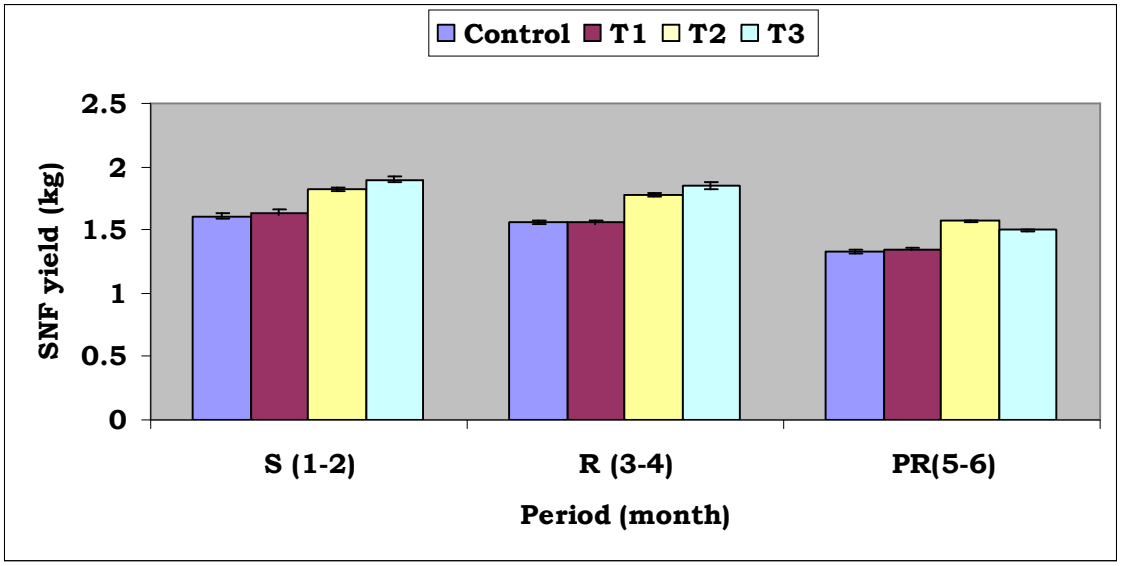


Figure 4.10
Average SNF yield (Kg/cow/day) of different treatment groups during different periods

4.3 EFFECT OF POLYHERBAL SUPPLEMENTATION ON IMMUNE STATUS OF COWS

4.3.1 Total Leukocyte Count (TLC)

TLC data presented in table 4.12 indicate no significant difference among treatments over different fortnights around calving. However, numerically TLC values at different time periods were highest for T_3, followed by T_2, T_1 and control. During prepartum, TLC values for all the groups increased towards calving and again during postpartum decreased away from calving. Similar to these Nazifi *et al.* (2008) also reported that TLC concentration increases towards parturition and decreases away from parturition. These results suggest that Polyherbal supplementation did not have any significant bearing on TLC concentration in the present study.

Table 4.13
Means (±SE) of total leucocytes count (thousands/ml) in different treatment groups

Period (days)	Treatment			
	Control	T_1 (150 g/kg/BW)	T_2 (200 mg/kg/BW)	T_3 (250 mg/kg/BW)
-30	8815±158.65	8490 ±229.71	8815±356.19	8885±246.87
-15	8960±165.46	8550±287.03	8830±248.42	9040±75.57
0	9270^{ab}±224.38	8550^{a}±248.33	8890^{a}±173.53	9585^{b}±173.69
15	9065±163.82	8580±229.15	8950±196.36	9220±136.44
30	8905^{a}±198.11	8530^{a}±243.95	8975^{ab}±160.25	9160^{b}±425.36

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

4.3.2 Differential Leukocyte Count (DLC)

Percent differential leukocyte count data are presented in table 4.13. Results reveal that at -30,-15 and 0 day of calving (prepartum period) lymphocyte % among treatment groups did not vary significantly ($p \leq 0.05$). However, at 15 and 30 days of calving, lymphocyte%, in T_1, T_2 and T_3 was significantly ($p \leq 0.05$) higher than control. In all treatment groups lymphocyte% showed significant depression towards calving and again as the cows moved away from calving date the lymphocyte percentage showed an increasing trend.

Percent neutrophil concentration among treatments, at -30,-15 and 0 days of calving (prepartum period) did not vary significantly ($p \leq 0.05$), however, neutrophil concentration was numerically higher for control group at -30 days and for T_3 at -15 and 0 day of calving. During postpartum period, at 15 days of lactation, neutrophils% in control was significantly ($p \leq 0.05$) higher than T_1, T_2 and T_3, however, there was no significant difference among T_1, T_2 and T_3. At 30 days of lactation no specific trends were observed for neutrophil%. Over the period, from -30 to 0 day of calving, neutrophils% increased significantly ($p \leq 0.05$) in all the groups and again decreased significantly ($p \leq 0.05$) after calving during lactation. Similar to these trends, Meglia *et al.*, (2001) and Kornmatitsuk *et al.,* (2001) also reported highest TLC and PMN cells at one day after calving whereas mononuclear cells increased 3 days after calving. As neutrophil serve as first line of defense against invasion of pathogenic microorganisms and play an important role in augmenting nonspecific immunity of host (Craven and Williams, 1985). Therefore increase in neutrophil% towards calving can be considered as host preparation to combat stress associated with fetus growth, parturition and negative energy balance. After calving higher neutrophils concentration can be associated with either production stress or sub-clinical or clinical infection. As milk production of control group was significantly lower than that of groups T_2 and T_3, therefore, in the absence of the higher production stress, the only plausible explanation for significantly ($p \leq 0.05$) higher neutrophil concentration in this group, at 15 days post calving,

might be the presence of high level of sub-clinical (Table 4.22, 4.23 and 4.14) and clinical (Table 4.25) infection.

Monocyte % among the groups did not vary significantly ($p \leq 0.05$) at different test days before and after parturition, however, contrary to findings of Meglia *et al.*, (2001) monocyte % in control and all treatment groups showed significant depression towards parturition. During post calving periods, monocyte % increased away from calving.

In relation to day of calving, eosinophil and basophil %, in control and all treatment groups did not show significant ($p \leq 0.05$) change. At 30 days pre-calving period there was no significant ($p \leq 0.05$) difference between control and different treatment groups, however, at 15 day prior to calving, eosinophil % in control group was significantly($p \leq 0.05$) higher than treatment groups and at 0 days of calving eosinophil % in control and T_1 was significantly($p \leq 0.05$) higher than T_2 and T_3. During postcalving period, no specific trend was observed; however, eosinophil % in control group was numerically higher than T_1, T_2 and T_3. During prepartum period on different test day basophil % did not show any specific trend for different groups and during post calving period, basophil % for different groups, did not reveal any significant variation.

Table 4.14
Least squares mean (±SE) of differential leukocytes count (DLC) (%) in different treatment groups

Type of cells	Period	Treatment			
		Control	T_1	T_2	T_3
Lymphocyte	-30	59.72^{D}±0.60	60.03^{C}±0.84	59.99^{C}±0.84	61.76^{C}±0.66
	-15	54.53^{Ca}±0.62	57.76^{BCb}±0.74	56.21^{Bab}±0.80	$54.72^{B\ a}$±0.45
	0	44.87^{A}±0.51	46.99^{A}±1.23	46.06A±0.64	44.53^{A}±0.49
	15	$49.75^{B\ a}$±0.88	$54.58^{B\ b}$±0.55	$53.72^{B\ b}$±0.50	54.58^{Bb}±0.47
	30	52.61^{BCa}±0.61	58.76^{BCc}±0.59	$56.24^{B\ b}$±0.60	54.21^{Bab}±0.51
Neutrophil	-30	35.62^{A}±0.75	35.13^{A}±1.13	35.30^{A}±0.69	33.92^{A}±0.71
	-15	40.26^{Bab}±0.87	37.77^{ABa}±0.77	39.63^{Bab}±0.85	41.57^{Bb}±0.47
	0	50.56^{Dab}±0.46	48.82^{Ca}±1.36	50.37^{Cab}±0.80	52.09^{Cb}±0.51
	15	45.74^{Cb}±1.06	41.00^{Ba}±0.59	42.15^{Ba}±0.63	41.29^{Ba}±0.42
	30	41.88^{Bc}±0.60	36.52^{Aa}±0.57	39.57^{ABb}±0.63	41.56^{Bc}±0.46
Monocyte	-30	2.45^{AB}±0.14	2.96^{B}±0.20	2.26^{B}±0.14	2.20^{AB}±0.22
	-15	2.47^{AB}±0.19	2.65^{AB}±0.10	2.50^{C}±0.12	2.00^{AB}±0.11
	0	2.14^{Ab}±0.21	1.99^{Aa}±0.12	1.92^{Aa}±0.09	1.71^{Aa}±0.10
	15	2.23^{A}±0.18	2.64^{AB}±0.09	2.51^{C}±0.19	2.50^{B}±0.11
	30	$3.15^{B\ b}$±0.21	$2.59^{AB\ b}$±0.18	2.25^{a}±0.13	$2.40^{AB\ a}$±0.13
Eosinophil	-30	1.52±0.13	1.20±0.12	1.32±0.10	1.22±0.05
	-15	1.60^{b}±0.11	1.14^{a}±0.10	1.20^{a}±0.06	1.15^{a}±0.14
	0	1.43^{b}±0.05	1.41^{b}±0.12	1.02^{a}±0.09	1.09^{a}±0.07
	15	1.40^{b}±0.11	1.13^{ab}±0.06	1.01^{a}±0.12	1.10^{ab}±0.09
	30	1.49^{b}±0.09	1.16^{ab}±0.08	1.10^{a}±0.08	1.23^{ab}±0.10
Basophil	-30	0.69^{a}±0.08	0.68^{a}±0.10	1.13^{b}±0.14	0.90^{a}±0.14
	-15	1.14^{b}±0.24	0.68^{b}±0.07	0.45^{a}±0.08	0.56^{a}±0.13
	0	1.01±0.18	0.79±0.12	0.63±0.11	0.59±0.11
	15	0.88±0.07	0.65±0.07	0.60±0.06	0.63±0.07
	30	0.88±0.09	0.96±0.11	0.83±0.10	0.60±0.13

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

4.3.3 Neutrophils Phagocytic Index

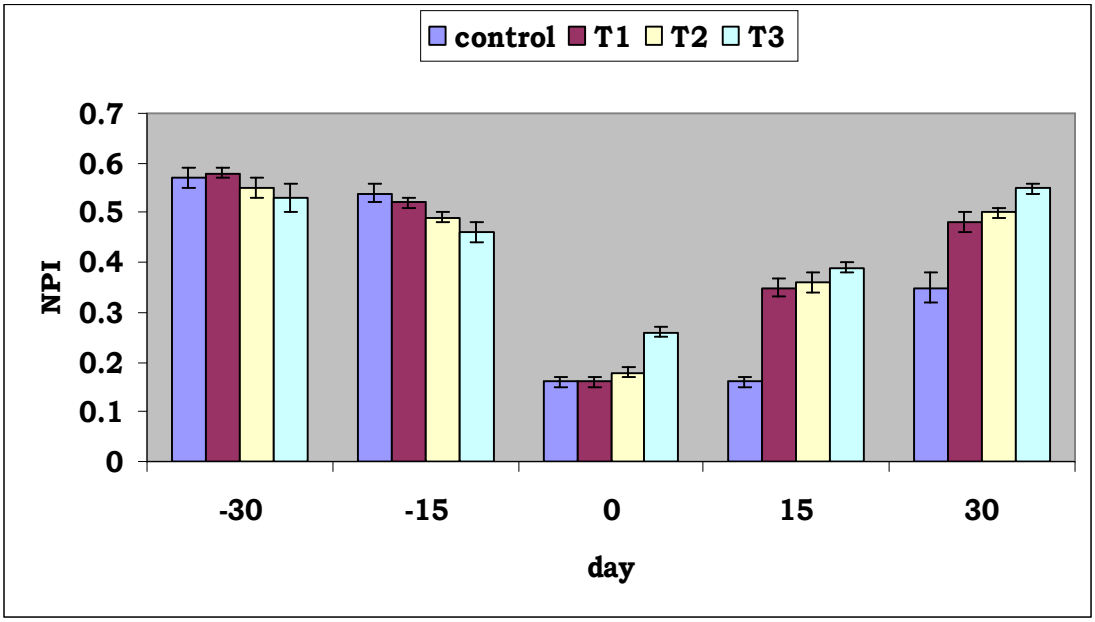
Neutrophils phagocytic index is an indicator of neutrophil phagocytic activity. The data for neutrophils phagocytic index are presented in table 4.14. This data reveal that at -30 day, there was no significant ($p \leq 0.05$) difference between control and different treatment groups, however, at -15 and 0 day of calving neutrophil phagocytic activity in T_3 was significantly($p \leq 0.05$) higher than control, T_1 and T_2, but no significant ($p \leq 0.05$) difference was observed among control, T_1 and T_2. During post caving period, at 15 and 30 day of lactation, neutrophil phagocytic activity in T_1, T_2 and T_3 was significantly($p \leq 0.05$) higher than control, but there was no significant ($p \leq 0.05$) difference among T_1, T_2 and T_3. Within the group, from -30 to 0 day of calving, neutrophil phagocytic activity in control as well as all the treatment groups decreased significantly ($p \leq 0.05$), but this depression was highest (72.24%) in T_1 followed by 71.92% in control, 67.27% in T_2 and 50.09% in T_3. While during post partum period, from 0 to 30 day of calving, neutrophil phagocytic activity in control as well all treatment groups increased significantly ($p \leq 0.05$), but this increase was highest, 66.66% in T_1 followed by 64.00% in T_2, 54.43% in control and 52.72% in T_3. These results suggest that phagocytic activity of neutrophils depressed in and around parturition (Kehrli *et al.*, 1989a, b). This depression might be due to decreased concentration of most of fat soluble antioxidant vitamins such as retinol, α-tocopherol and β-carotene at the time of parturition (Batra *et al.*, 1992,Michal *et al.*, 1994,Weiss *et al.*, 1997 and Weiss,1998). These vitamins increase intracellular kill activity of PMN (Hogan *et al.*, 1993, Chew, 1996, Sato, 1998 and Weiss *et al.* 1998. These vitamins improve cellular and humoral immune function due to chain breaking lipid soluble tissue antioxidant properties (Halliwell, 1987). Therefore, it can be inferred from the results that polyherbal supplementation improved neutrophil phagocytic activity and its effect can be attributed to antioxidant property (Table 4.15) of its ingredients.

Table 4.15
Means (±SE) of neutrophil phagocytic index in different treatment groups

Period (days)	Treatment			
	Control	T_1	T_2	T_3
-30	0.57^{C}±0.02	0.58^{D}±0.01	0.55^{C}±0.02	0.53^{CD} ±0.03
-15	$0.54^{C\,a}$ ±0.02	$0.52^{CD\,a}$±0.01	$0.49^{C\,a}$ ±0.01	$0.46^{BC\,b}$±0.02
0	$0.16^{A\,a}$ ±0.01	$0.16^{A\,a}$ ±0.01	$0.18^{A\,a}$ ±0.01	$0.26^{A\,b}$ ±0.01
15	$0.16^{A\,a}$ ±0.01	$0.35^{B\,b}$ ±0.02	$0.36^{B\,b}$ ±0.02	$0.39^{B\,b}$ ±0.02
30	$0.35^{B\,a}$ ±0.03	$0.48^{C\,b}$ ±0.02	$0.50^{C\,b}$ ±0.01	$0.55^{D\,b}$ ±0.01

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Figure 4.11
Neutrophil phagocytic activity at different test days in different treatment groups



4.3.4 Total Plasma Antioxidant Activity

The total plasma antioxidant activity is an indicator of stress to animal. This was evaluated by determining ferric reducing antioxidant power (FRAP). The results for FRAP (Table 4.15) reveal that during prepartum period, at -30th and -15th day, the value of FRAP (µmole/L) for control and different treatments did not differ significantly ($p \leq 0.05$) but FRAP value for T_3 group were numerically higher, followed by T_2, control and T_1. At calving day FRAP value for T_3 group was significantly ($p \leq 0.05$) higher than control, T_1 and T_2, however, there was no significant ($p \leq 0.05$) difference among control, T_1 and T_2. During post partum period, at 15th and 30th test day of lactation, FRAP value for T_3 group was significantly ($p \leq 0.05$) higher than control and T_1, however, there was no significant ($p \leq 0.05$) difference among control, T_1 and T_2 and between T_2 and T_3.

Over the period within the group, from -30th to 0 day of calving, FRAP value decreased in control and all treatment, however, this depression was significant ($p \leq 0.05$) only in control. From 0 to 30th day post calving, again FRAP value increased in control and all treatment groups, but, this increase was significant ($p \leq 0.05$) only in control. These results indicate that from -30th to 0 day of calving FRAP value depression in control and T_1 was higher than T_2 and T_3. Therefore, cows in T_2 and T_3 had better antioxidant status and experienced lesser stress. This effect of polyherbal preparation can be attributed to antioxiadant property of its individual ingredients, *Ocimum sanctum* (Geetha and Vasudevan, 2004), *Emblica officinalis* (Kumaran *et al.*, 2006), *Withania somnifera* (Bhattacharya *et al.*, 1987), *Asparagus racemosu*s (Visavadiya and Narasimhacharya, 2005), *Tribulus terrestris* (Amin *et al.*, 2006) and *Tinospora cordfolia* (Prince and Menon, 1999).

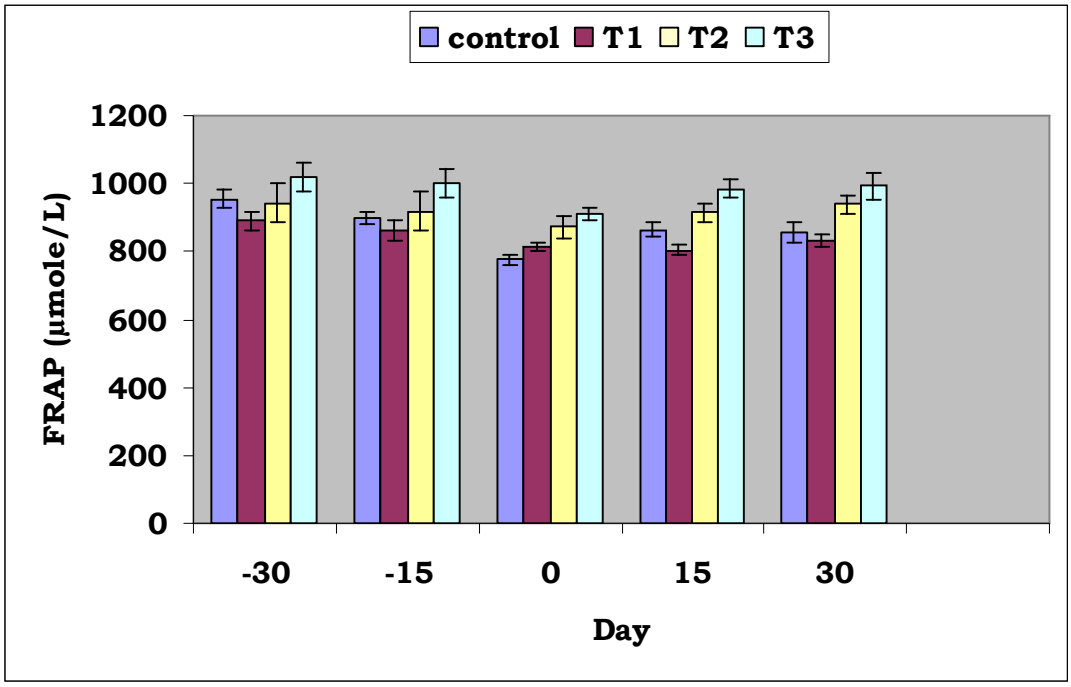
Table 4.16

Means (±SE) of ferric reducing antioxidant power (FRAP) (µmole/L) in different treatment groups

Period (days)	Treatment			
	Control	T_1	T_2	T_3
-30	953.33^{B} ±27.15	890.73^{A} ±26.64	941.63^{A} ±56.44	1017.24^{A} ±42.61
-15	900.16^{B} ±18.60	865.04^{A} ±30.20	919.02^{A} ±56.98	998.70^{A} ±42.04
0	$775.61^{A\,a}$ ±17.09	$814.96^{A\,a}$ ±13.31	$872.52^{A\,a}$ ±31.80	$909.99^{A\,b}$ ±18.33
15	$864.88^{B\,a}$ ±21.02	$803.90^{A\,a}$ ±15.24	$914.15^{A\,ab}$ ±27.76	$985.85^{A\,b}$ ±24.68
30	$856.59^{B\,a}$ ±30.18	$834.80^{A\,a}$ ±18.44	$937.89^{A\,ab}$ ±28.28	993.01^{Ab} ±37.35

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Figure 4.12
FRAP at different test days in different treatment groups



4.3.5 Non-Esterified Fatty Acid (NEFA) Concentration

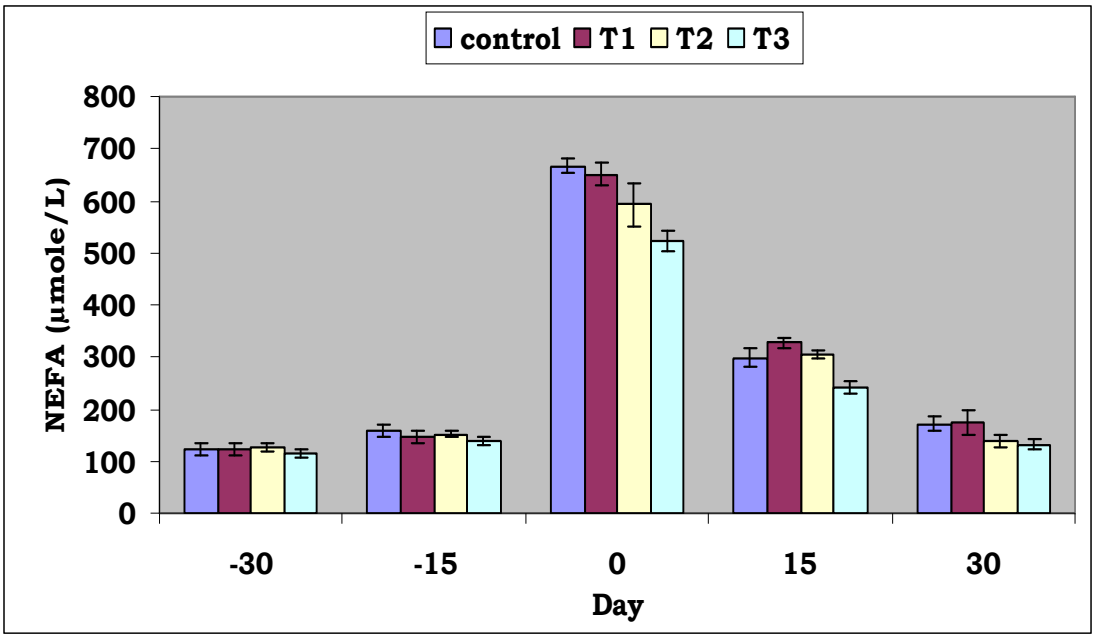
Higher plasma NEFA concentration is an indicator of negative energy balance of animals (Grummer *et al.,* 2004). Therefore plasma NEFA concentration indirectly reflects dry matter and feed intake of animals. The values of NEFA (µmole/L) are presented in table 4.16. The results reveal that during prepartum, at -30th and -15th test day, there was no significant ($p \leq 0.05$) difference in NEFA concentration among control and different treatment groups, however, NEFA values were highest for control and lowest for T_3. On 0 and 15th day of calving, NEFA concentration in T_3 was significantly ($p \leq 0.05$) lower than control, T_1 and T_2, but, there was no significant ($p \leq 0.05$) difference among control, T_1 and T_2. However, on 30th day of calving, NEFA concentration in both, T_2 and T_3 was significantly ($p \leq 0.05$) lower than control and T_1. Over the period within the group, plasma NEFA concentration in control and all treatment groups increased significantly ($p \leq 0.05$) towards calving and again during postpartum period decreased away from calving. From -30th day to calving, increase in plasma NEFA concentration was highest 81.44% in control followed by 80.94% in T_1, 78.80% in T_2 and 78.04% in T_3. These results indicate that during periparturient period, In comparison to T_3 and T_2, cows in control and T_1 were in higher negative energy balance (Doepel *et al.,* 2002). It was reflected in lower milk yield (Table 4.2) for these groups. This higher negative energy balance could be associated to poor dry matter and feed intake (Dann *et al.* 2005). Therefore from lower concentration of NEFA in and around calving in T_2 and T_3, it can be concluded that polyherbal immunomodulator supplementation prevented feed intake depression during periparturient period.

Table 4.17
Means (±SE) of NEFA (µmole/L) in different treatment groups

Period (days)	Treatment			
	Control	T_1	T_2	T_3
-30	123.65^{A} ±11.63	124.07^{A} ±11.81	125.61^{A} ±7.88	114.66^{A} ±6.26
-15	157.55^{A} ±11.39	145.99^{A} ±12.93	152.14^{A} ±6.54	138.59^{A} ±7.85
0	$666.25^{C\,b}$ ±14.31	$651.27^{C\,b}$ ±21.43	$592.71^{C\,b}$ ±40.64	$522.31^{C\,a}$ ±20.35
15	$298.09^{B\,b}$ ±16.89	$328.36^{B\,b}$ ±9.82	$304.50^{B\,b}$ ±6.52	$242.27^{B\,a}$ ±10.97
30	$172.27^{A\,c}$ ±12.22	174.89^{Ac} ±22.84	140.27^{Ab} ±11.95	$132.14^{A\,a}$ ±9.98

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Figure 4.13
Plasma NEFA concentration at different test days in Different treatment groups



4.3.6 Plasma Immunoglobulin-G (Ig G) Concentration

The results for Ig G (mg/ ml) are presented in table 4.17. Results reveal that, during prepartum period, plasma Ig G concentration in T_3 and T_2 at -30th and -15th day of calving were significantly ($p \leq 0.05$) higher than control and T_1, However there was no significant difference between T_3 and T_2 and between control, T_1 and T_2. At 0 day of calving, Ig G concentration in T_1 was significantly ($p \leq 0.05$) lower than control, T_2 and T_3, but there was no difference between control, T_2 and T_3.

During postpartum period, at 15th test day Ig G concentration in T_3 was significantly ($p \leq 0.05$) higher than control, T_1 and T_2, but there was no significant difference among control, T_1 and T_2. However, at 30th test day, Ig G concentration in T_3 was significantly ($p \leq 0.05$) higher than T_2 only, but no significant difference was observed between control, T_1 and T_3.

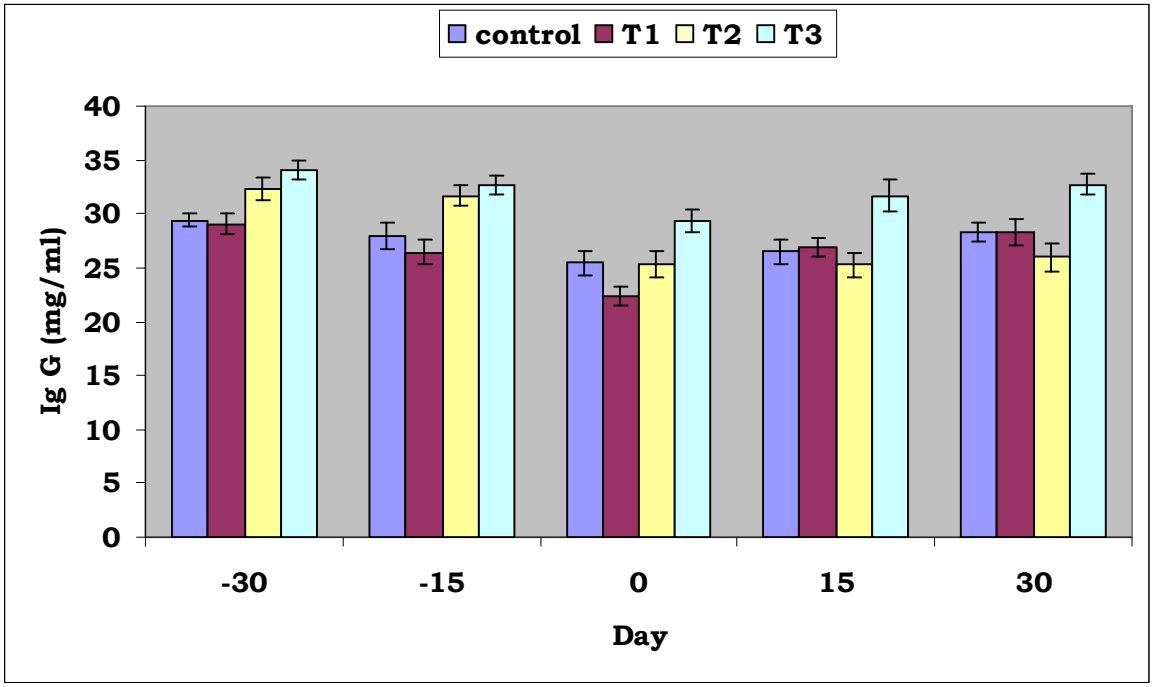
Similar to Detilleux *et al.* (1995) and Kehrli *et al.* (1998b) in control as well as different treatments, Ig G concentration declined toward parturition but it was significant ($p \leq 0.05$) only in T_1 and T_2. As immunoglobulins are secreted from B-lymphocytes, therefore, diminished lymphocyte responsiveness around calving (Kehrli *et al.* 1989a and Saad *et al.* 1989) results in to declined IgG concentration. It can be inferred from these results that cows in treatment group T_2 and T_3 had better immune status during peripartum period. These results were in line with the findings of Acharya *et al.* (2002). They found significant increases in ALC and IgG level in mastitis affected cow after supplementation of a polyherbal preparation, immu-21, containing *Ocimum sanctum, Emblica officinalis, Withania Somnifera* and *Tinospora cordifolia*.

Table 4.18
Means (±SE) of Ig G (mg/ml) in different treatment groups

Period (days)	Treatment			
	Control	T_1	T_2	T_3
-30	$29.40^{A\ a}$ ±0.59	$29.03^{B\ a}$ ±0.97	$32.29^{B\ ab}$ ±1.00	$34.40^{A\ b}$ ±0.89
-15	$27.94^{A\ a}$ ±1.18	$26.43^{AB\ a}$ ±1.12	$31.66^{B\ ab}$ ±0.93	$33.57^{A\ b}$ ±0.82
0	$25.43^{A\ ab}$±1.16	$22.36^{A\ a}$ ±0.93	$25.28^{A\ ab}$ ±1.21	$30.10^{A\ b}$ ±1.07
15	$26.51^{A\ a}$ ±1.12	$26.94^{B\ a}$ ±0.91	$25.26^{A\ a}$ ±1.15	$32.44^{A\ b}$ ±0.49
30	$28.24^{A\ ab}$ ±0.87	$28.30^{B\ ab}$ ±1.16	$25.95^{A\ a}$ ±1.38	$32.74^{A\ b}$ ±0.94

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Figure 4.14
Plasma Ig G concentration at different test days in different treatment groups



4.3.7 Plasma Micro-minerals Concentration

4.3.7.1 Plasma Copper (Cu) Concentration

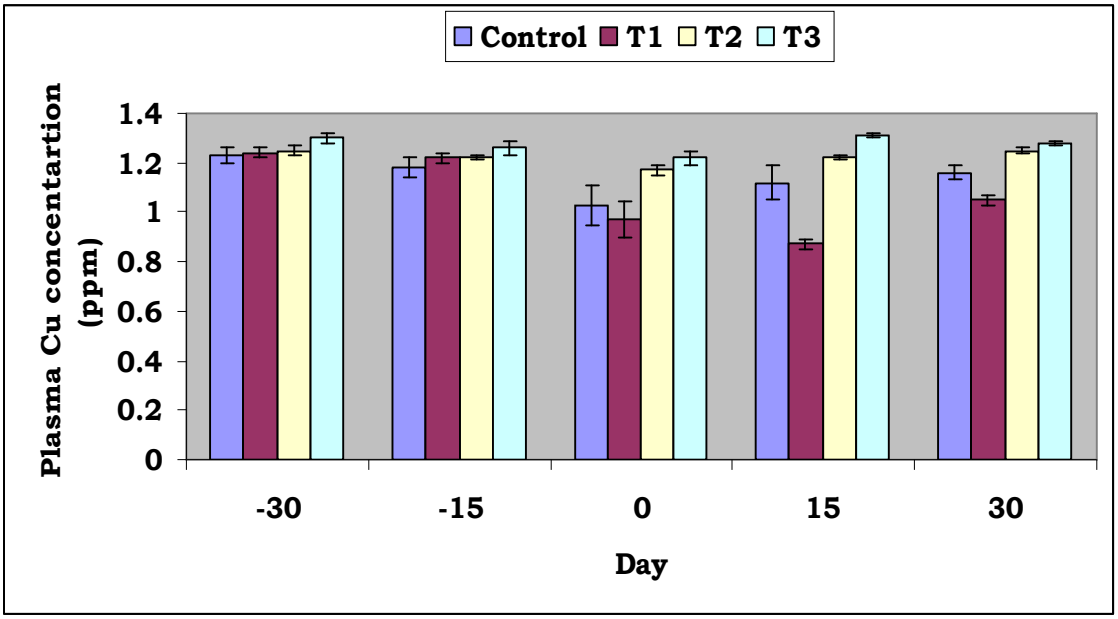
The results for plasma Cu (ppm) concentration (Table 4.18) reveal that at -30th and -15th test day of calving, plasma Cu (ppm) concentration among control and different treatments did not differ significantly ($p \leq 0.05$), however, Cu concentration was numerically higher for T_3 followed by T_2, T_1 and control, in decreasing order. At the calving day, Cu concentration in T_3 and T_2 was significantly ($p \leq 0.05$) higher than control and T_1, but no significant difference was observed between T_2 and T_3. During postpartum period, at 15th and 30th test day, Cu concentration in T_1 were significantly ($p \leq 0.05$) lower than control, T_2 and T_3, but no difference was observed among control, T_2 and T_3. Within the group, plasma Cu concentration in control and T_1, from -30th to 0 day of calving, decreased significantly ($p \leq 0.05$), but over this period no significant depression was observed in T_2 and T_3. After calving, from 0 to 30th day of calving, plasma Cu concentration in control and T_1, again showed significant ($p \leq 0.05$) increase. In healthy cows, Durand and Kawashima (1979) and Singh *et al.* (1991) reported slight increase in plasma Cu concentration during prepartum period, however, similar to Bostedt *et al.* (1974) and Rajora and Pachuri (1994), in T_2 and T_3 group plasma Cu concentration did not depress significantly. Low plasma cu concentration can be due to either reduced dietary uptake or deviation of plasma Cu for the increased synthesis of ceruloplasmin, an acute phase protein during disease (Broadley and Hoover, 1989). Therefore significant depression in plasma Cu concentration in control and T_1 could be attributed to higher feed intake depression in control group, indicated by higher plasma NEFA value (Table 4.16) and high incidence of clinical mastitis (Table 4.25).

Table 4.19
Means (±SE) of copper (ppm) in different treatment groups

Period (days)	Treatment			
	Control	T_1	T_2	T_3
-30	1.23^{B}±0.03	1.24^{C} ±0.02	1.25±0.02	1.30±0.02
-15	1.18^{AB} ±0.04	1.22^{B}±0.02	1.22±0.01	1.26±0.03
0	$1.03^{A\ b}$ ±0.08	$0.97^{A\ a}$ ±0.07	1.17^{c} ±0.02	1.22^{c}±0.03
15	$1.12^{AB\ b}$ ±0.07	$0.87^{A\ a}$ ±0.02	1.22^{b} ±0.01	1.31^{b} ±0.01
30	1.16^{AB} ^{b}±0.03	$1.05^{AB\ a}$ ±0.02	1.25^{b} ±0.01	1.28^{b} ±0.01

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Figure 4.15
Plasma Cu concentration at different test days in different treatment groups



4.3.7.2 Plasma Iron (Fe) Concentration

The results for plasma Fe (ppm) concentration (Table 4.19) show that at -30th and -15th test day of calving, plasma Fe (ppm) concentration among control and different treatments did not differ significantly ($p \leq 0.05$), however, Fe concentration was numerically higher in T_3 and T_2 than T_1 and control. At calving day, Fe concentration in control was significantly ($p \leq 0.05$) lower than T_1, T_2 and T_3 but no significant difference was observed among T_1, T_2 and T_3. Within the group, Fe concentration in control as well as different treatments did not show significant depression towards calving. Similar to these results Soliman and Amrousi (1965) also reported decrease in plasma iron concentration towards parturition because of increased fetal demand.

Figure 4.16
Plasma iron concentration at different test days in different treatment groups

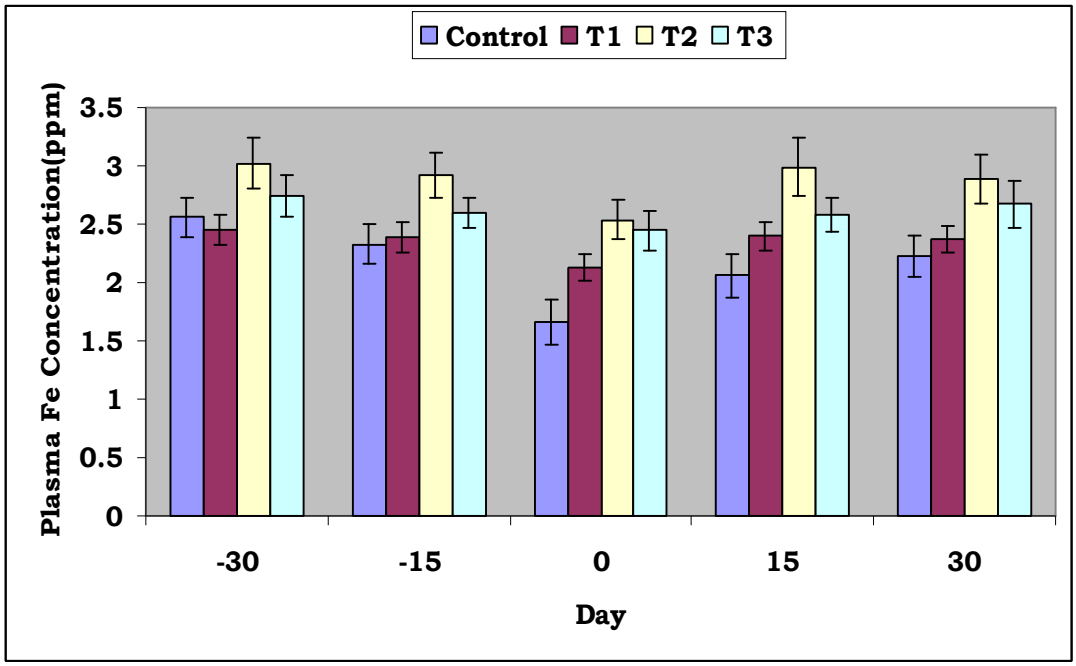


Table 4.20
Means (±SE) of iron (ppm) in different treatment groups

Period (days)	Treatment			
	Control	**T_1**	**T_2**	**T_3**
-30	2.56±0.17	2.45±0.13	3.02±0.22	2.74±0.18
-15	2.33±0.17	2.39±0.13	2.92±0.19	2.59±0.13
0	1.66^{a} ±0.19	2.13^{b} ±0.11	2.54^{b} ±0.17	2.45^{b} ±0.17
15	2.06 ±0.19	2.40 ±0.12	2.99 ±0.25	2.58 ±0.14
30	2.23±0.18	2.37±0.11	2.88±0.21	2.67±0.20

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

4.3.7.3 Plasma Manganese (Mn) concentration

The results for plasma Mn (ppm) concentration (Table 4.20) reveal no significant (p≤0.05) difference among control and different treatments at various test days before and after calving, but at various test days plasma Mn (ppm) concentration in T_3 were numerically higher than control, T_1 and T_2. Within the group, Mn concentration in none of the group depressed significantly in and around calving. Setia *et al.* (1994) suggested that the slight decrease in Mn during the late pregnancy might be due to increased utilization of glucosyl transferases for the synthesis of mucopolysaccharides. These mucopolysaccharides are vital structural components of cartilage and are thereby utilized for the development of fetal cartilage.

Figure 4.17

Plasma manganese concentrations at different test days in different treatment groups

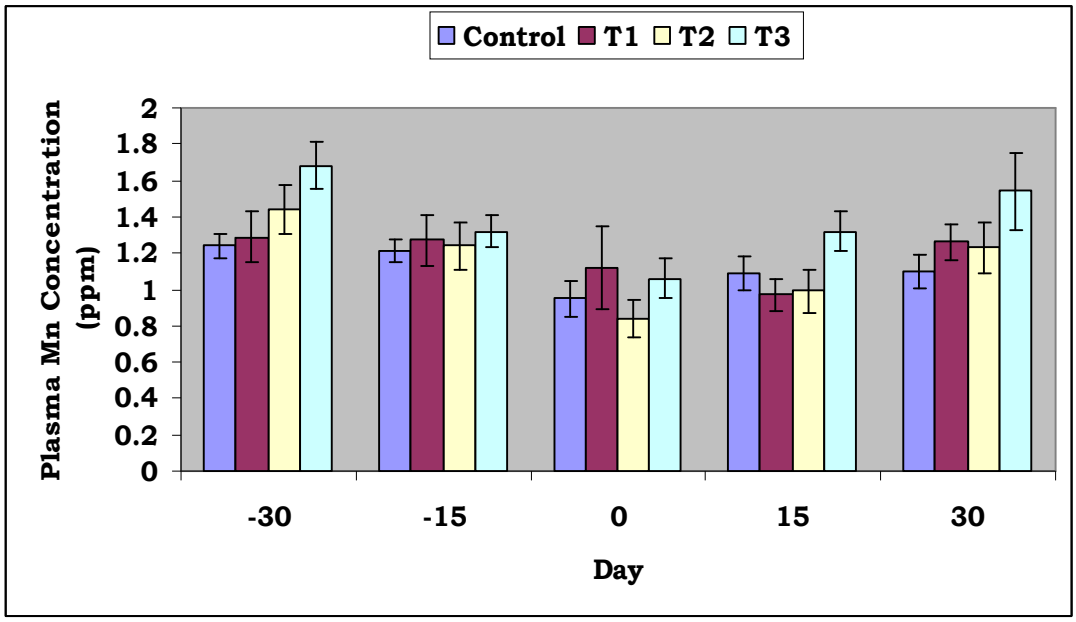


Table 4.21

Means (±SE) of manganese (ppm) in different treatment groups

Period (days)	Treatment			
	Control	T_1	T_2	T_3
-30	1.24±0.07	1.29±0.14	1.44±0.13	1.68±0.13
-15	1.21±0.06	1.27±0.14	1.24±0.13	1.32±0.09
0	0.95±0.10	1.12±0.23	0.84±0.10	1.06±0.11
15	1.09±0.09	0.97±0.09	0.99±0.12	1.32±0.11
30	1.10±0.09	1.26±0.10	1.23±0.14	1.54±0.21

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

4.3.7.4 Plasma Zinc (Zn) Concentration

The results of plasma Zn (ppm) concentration are presented in table 4.21. It is evident from the results that at -30th and -15th test day of calving, plasma Zn (ppm) concentration among control and different treatments did not differ significantly ($p \leq 0.05$), however, Zn concentration was highest for T_3 followed by T_2, control and T_1. At the calving day, Zn concentration in control was significantly ($p \leq 0.05$) lower than T_1, T_2 and T_3 but no difference was observed between T_1, T_2 and T_3. Within the group, only in control, Zn concentration decreased significantly ($p \leq 0.05$) towards calving. These findings are in agreement with those reported by Goff and Stabel (1990) and Xin *et al.*, (1993). They suggested that blood Zn level declines around calving due to reduced DMI, transfer of Zn to colostrum and increased stress at this time. Therefore, In comparison to treatments, higher decline of blood Zn concentrations in control can be attributed to high degree of parturition stress and incidence of clinical mastitis. As parturition stress and microbial infections causes redistribution of Zn from blood to tissues, especially the liver (Spears *et al.*, 1991; Underwood and Suttle, 1999).

Figure 4.18
Plasma zinc concentration at different test days in different treatment groups

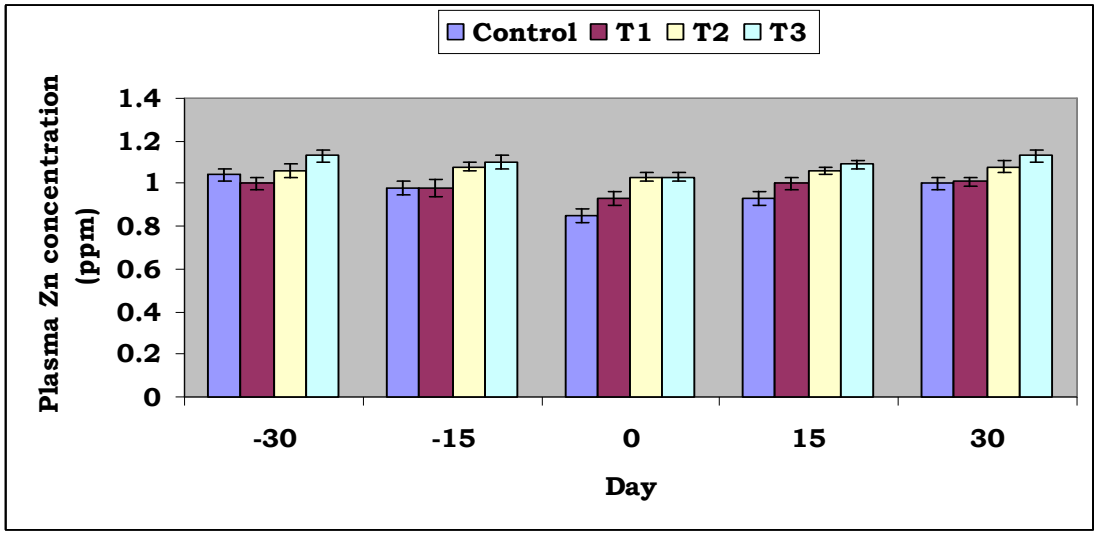


Table 4.22
Means (±SE) of zinc in different treatment groups

Period (days)	Treatment			
	Control	T_1	T_2	T_3
-30	1.04^{B} ±0.03	1.00 ±0.03	1.06 ±0.03	1.13 ±0.03
-15	0.98^{B} ±0.03	0.98 ±0.04	1.08 ±0.02	1.10 ±0.03
0	$0.85^{A\,a}$ ±0.03	0.93^{b} ±0.03	1.03^{b} ±0.02	1.03^{b} ±0.02
15	0.93^{AB} ±0.03	1.00 ±0.03	1.06 ±0.02	1.09 ±0.02
30	1.00^{B} ±0.03	1.01 ±0.02	1.08 ±0.03	1.13 ±0.03

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

4.4 UDDER HEALTH AND MILK QUALITY

4.4.1 Milk Somatic Cell Count

Fortnightly average values of milk somatic cell count (SCC) are presented in Table 4.22. Results reveal that mean value of milk SCC (x10^5cells/ml) for different fortnights did not differ significantly (p≤0.05), however, mean SCC for control and T_1 were on numerically higher sides and for T_2 and T_3 were on lower sides. But overall mean values of SCC in T_2 and T_3 was significantly (p≤0.05) lower than control and T_1, however, there was no significant difference between control and T_1. These results suggest that polyherbal supplementation during prepartum reduced somatic cell counts in milk during early lactation. Lower mean value of SCC in higher supplemented group could be attributed to better udder health (Dohoo and Leslie, 1991), indicated by low CMT score (Table 4.24)

and no incidence of clinical mastitis (Table 4.25) in these groups. However, the cows with elevated level of SCC in early stage of lactation indicates presence of intramammary infection (IMI) around calving and such cows are more prone (1.5 times higher chances) to suffer from clinical mastitis in subsequent days of lactation (Suriyasathaporn *et al.*, 2000). In this study also, maximum % of cows affected with mastitis were from control and T_1.

Table: 4.23 Least squares analysis of variance for immunity parameters in different treatments at different periods

Parameter	MSS Period (4)	MSS Treatment (3)	MSS Period x Treatment (12)	MSS Error (180)
TLC	570143.75	3625245.83*	159110.42	523915.28
FRAP	63717.51*	183267.75*	577124	10036.63
NEFA	1646727.43*	32829.81*	7234.12*	2482.66
Phagocytic activity	1227.32	1213.43	1197.29	1203.75
Cu	0.16*	0.40*	0.05*	0.01
Fe	1.35*	4.68*	0.14*	0.28
Mn	1.10*	0.72*	0.11	0.16
Zn	0.06*	0.20*	0.01	0.01

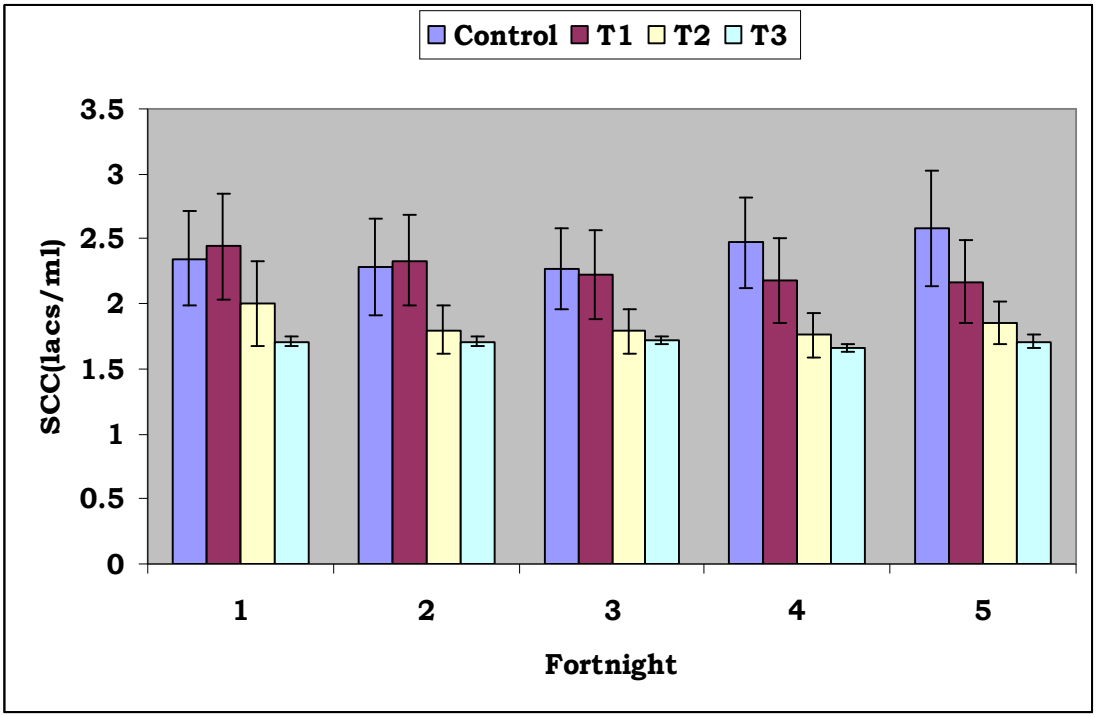
- Significant at 1% level of significance
- Figures in parenthesis indicate degree of freedom

Table 4.24
Means (±SE) of somatic cell count (SCC) (10^5/ml) in different treatment groups

Fortnight	Treatment			
	Control	T_1	T_2	T_3
1	2.35±0.37	2.44±0.41	2.00±0.33	1.71±0.04
2	2.28±0.37	2.33±0.35	1.80±0.18	1.71±0.04
3	2.27±0.31	2.22±0.34	1.79±0.17	1.72±0.03
4	2.47±0.35	2.18±0.32	1.76±0.17	1.66±0.03
5	2.58±0.44	2.17±0.32	1.85±0.16	1.71±0.05
Overall	**2.39^{c}±0.12**	**2.27^{cb}±0.12**	**1.84^{b} ±0.12**	**1.70^{ab} ±0.12**

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Figure 4.19
Milk SCC (lacs/ml) of treatment groups during different fortnights



4.4.2 Milk Standard Plate Count

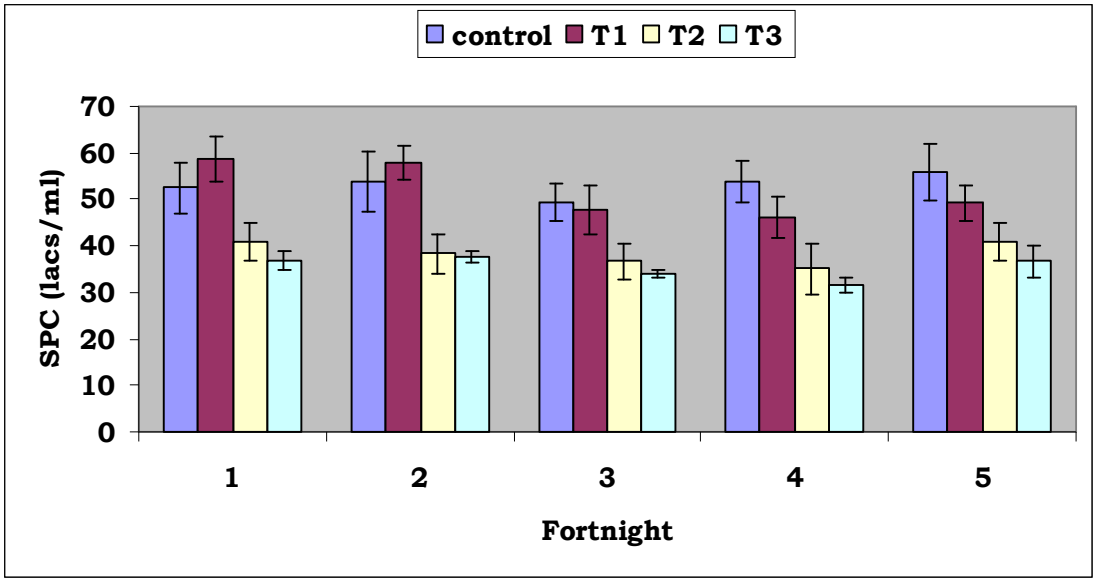
Standard plate count (SPC) denotes bacterial contamination of milk. Therefore SPC is an indicator of udder health and milk quality for human consumption. SPC data presented (Table.4.23) show that mean values of SPC for different fortnights were highest in control group, followed by T_1, T_2 in decreasing order. Average of mean SPC value for all fortnight in control, T_1, T_2 and T_3 was 53.08, 51.96, 38.36 and 35.30 x 10^5/ml, respectively. Mean values of SPC in T_2 and T_3 were significantly ($p \leq 0.05$) lower than for control and T_1. However, there was no significant difference, in mean SPC values between control and T_1 and between T_2 and T_3. Over the fortnights, no significant ($p \leq 0.05$) changes were observed in the different treatment groups. These results suggest that polyherbal supplementation improved milk quality through reducing milk bacterial load. These results of low SPC in T_2 and T_3 can be explained on the basis of low incidence of sub-clinical (Table 4.24) and clinical mastitis (Table 4.25) in these groups.

Table 4.25
Means (±SE) of standard plate counts (SPC) (lakhs/ml) in different treatment groups

Fortnight	Treatment			
	Control	T_1	T_2	T_3
1	$52.50^{A\ bc}$±5.44	$58.70^{A\ b}$±4.96	$40.80^{A\ a}$ ±4.15	$36.70^{A\ a}$ ±2.09
2	$53.90^{A\ bc}$ ±6.59	$57.90^{A\ c}$ ±3.63	$38.30^{A\ ab}$ ±4.20	$37.50^{A\ a}$ ±1.24
3	$49.40^{A\ b}$±4.16	$47.80^{A\ ab}$ ±5.13	$36.70^{A\ ab}$ ±3.84	$34.10^{A\ a}$ ±0.80
4	$53.90^{A\ b}$ ±4.36	$46.20^{A\ ab}$ ±4.46	$35.10^{A\ a}$ ±5.45	$31.50^{A\ a}$ ±1.73
5	$55.70^{A\ b}$±6.13	$49.20^{A\ b}$ ±3.94	$40.90^{A\ a}$ ±3.93	$36.70^{A\ a}$±3.44

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Figure 4.20
Average milk SPC (lacs/ml) of treatment groups during different fortnights



4.4.3 Sub-clinical Mastitis

Incidence and severity of sub-clinical mastitis was diagnosed through California Mastitis Test (CMT) score method. The results for CMT score for different treatment groups are presented in table 4.24. Percent incidence of sub-clinical mastitis for different fortnights for control, T_1, T_2 and T_3 ranged from 25.00-42.50, 27.50-40.00, 7.50-22.50 and 10.00-20.00%, respectively. Out of this, percentage of severely affected quarters over different fortnights for control, T_1 and T_2 ranged from 5.00-12.50, 7.50-12.50 and 0-2.50, respectively, however in T_3 group none of the quarter was severely affected. Average CMT score, indicating overall severity of sub-clinical mastitis in different groups, for different fortnights for control, T_1, T_2 and T_3 ranged from 0.72-1.30, 0.60-1.00, 0.50-1.50 and 0.30-0.50, respectively. These findings suggest that in comparison to T_1 and T_2, cows in control and T_1 groups showed higher incidence of severely affected sub-clinical mastitis. Therefore it can be inferred from these results that polyherbal supplementation at higher dose rate improved udder health. The better udder health of cows in higher polyherbal supplemented groups can be attributed to better immune status, indicated by higher neutrophil phagocytic activity (Cai *et al.*,1994) (Table 4.14), better antioxidant status (Goff, 2000) (Table 4.15), lower NEFA concentration (Klucinski *et al.*,1988) (Table 4.16) and higher IgG concentration(Table 4.17) in higher supplemented groups.

Table 4.26
Incidence of sub-clinical mastitis in different treatment groups

Period	Treatment	Level	Quarter				% incidence	Total incidence (%)	Average CMT score
			LF	RF	LH	RH			
1.	**Control**	Healthy	8	8	5	6	67.50	32.50	0.72
		Mild	1	1	2	1	12.50		
		Moderate	1	0	2	2	12.50		
		Severe	0	1	1	1	7.50		
	T_1	Healthy	7	8	6	6	67.50	32.50	1.00
		Mild	0	0	3	1	10.00		
		Moderate	2	1	0	1	10.00		
		Severe	1	1	1	2	12.50		
	T_2	Healthy	9	9	8	7	82.50	17.50	0.90
		Mild	1	1	1	0	7.50		
		Moderate	0	0	1	2	7.50		
		Severe	0	0	0	1	2.50		
	T_3	Healthy	10	10	7	5	80	20.00	0.40
		Mild	0	0	3	5	20		
		Moderate	0	0	0	0	0		
		Severe	0	0	0	0	0		
2.	**Control**	Healthy	6	7	7	5	62.50	37.50	1.20
		Mild	2	2	0	2	15.00		
		Moderate	2	0	2	3	17.50		
		Severe	0	1	1	0	5.00		
	T_1	Healthy	7	7	5	5	60.00	40.00	1.00
		Mild	2	2	1	0	12.50		
		Moderate	0	0	4	4	20		
		Severe	1	1	0	1	7.50		
	T_2	Healthy	9	9	9	4	77.50	22.50	0.70
		Mild	1	1	0	6	20		
		Moderate	0	0	1	0	2.5		
		Severe	0	0	0	0	0		
	T_3	Healthy	10	9	10	3	80	20.00	0.50
		Mild	0	1	0	7	20		
		Moderate	0	0	0	0	0		
		Severe	0	0	0	0	0		
3.	**Control**	Healthy	7	7	8	8	75.00	25.00	1.30
		Mild	0	2	0	0	5.00		
		Moderate	2	0	1	2	12.50		
		Severe	1	1	1	0	7.50		
	T_1	Healthy	6	7	8	8	72.50	27.5O	0.60
		Mild	1	1	1	1	10.00		
		Moderate	2	1	1	0	10.00		
		Severe	1	1	0	1	7.50		
	T_2	Healthy	10	10	9	5	85.00	17.50	0.50
		Mild	0	0	0	4	10.00		
		Moderate	0	0	1	2	7.50		
		Severe	0	0	0	0	0.00		
	T_3	Healthy	10	10	8	8	90.00	10.00	0.40

		Mild	0	0	2	1	7.50		
		Moderate	0	0	0	1	2.50		
		Severe	0	0	0	0	0.00		
4.	**Control**	Healthy	6	7	6	4	57.50	42.50	1.20
		Mild	1	2	1	3	17.50		
		Moderate	1	0	1	3	12.50		
		Severe	2	1	2	0	12.50		
	T_1	Healthy	7	9	6	6	70.00	30.00	0.70
		Mild	2	0	1	1	10.00		
		Moderate	0	0	3	2	12.50		
		Severe	1	1	0	1	7.50		
	T_2	Healthy	10	10	6	6	80.00	20.00	1.50
		Mild	0	0	3	4	17.50		
		Moderate	0	0	0	0	0.00		
		Severe	0	0	1	0	2.50		
	T_3	Healthy	10	10	8	8	90.00	10.00	0.30
		Mild	0	0	2	2	10.00		
		Moderate	0	0	0	0	0.00		
		Severe	0	0	0	0	0.00		
5.	**Control**	Healthy	6	7	5	6	60.00	40.00	1.10
		Mild	2	1	2	2	17.50		
		Moderate	1	0	1	2	10.00		
		Severe	1	2	2	0	12.50		
	T_1	Healthy	6	9	6	8	72.50	27.50	0.60
		Mild	3	0	0	0	7.50		
		Moderate	0	0	4	1	12.50		
		Severe	1	1	0	1	7.50		
	T_2	Healthy	10	10	7	10	92.50	7.50	0.50
		Mild	0	0	3	0	7.50		
		Moderate	0	0	0	0	0.00		
		Severe	0	0	0	0	0.00		
	T_3	Healthy	9	10	8	7	85.00	15.00	0.40
		Mild	1	0	1	3	12.50		
		Moderate	0	0	1	0	2.50		
		Severe	0	0	0	0	0		

4.5 INCIDENCE OF POSTPARTUM DISORDERS

Data of incidence of post parturient disorders (Table 4.25) indicate that maximum 40% of cows in control, followed by 30% in T_1 and 20% in T_2 suffered from clinical mastitis. However, in T_3 none of the cows suffered from clinical mastitis. The incidence of retained placenta in control, T_1, T_2 and T_3 was 10, 10, 20 and10%, respectively, and incidence of metritis in all groups was 10%. In control, T_1 and T_2 all the cows who suffered with metritis also developed clinical mastitis, whereas in T_3 none of the cow which suffered with metritis showed clinical mastitis. This better health of animals in T_2 and T_3 groups could be attributed to better antioxidant (Table 4.15) and immunity (Table 4.14 and 4.17) status of cows in these groups. Therefore, it can be concluded that polyherbal supplementation reduced the incidence of post parturient complication through improved immunity and reduced periparturient stress on the animals.

Table 4.27
Incidence (%) of periparturient disorders in different treatment groups

Disorder	Treatment			
	Control	T_1	T_2	T_3
Clinical mastitis	40	30	20	0
ROP	10	10	20	10
Metritis	10	10	10	10

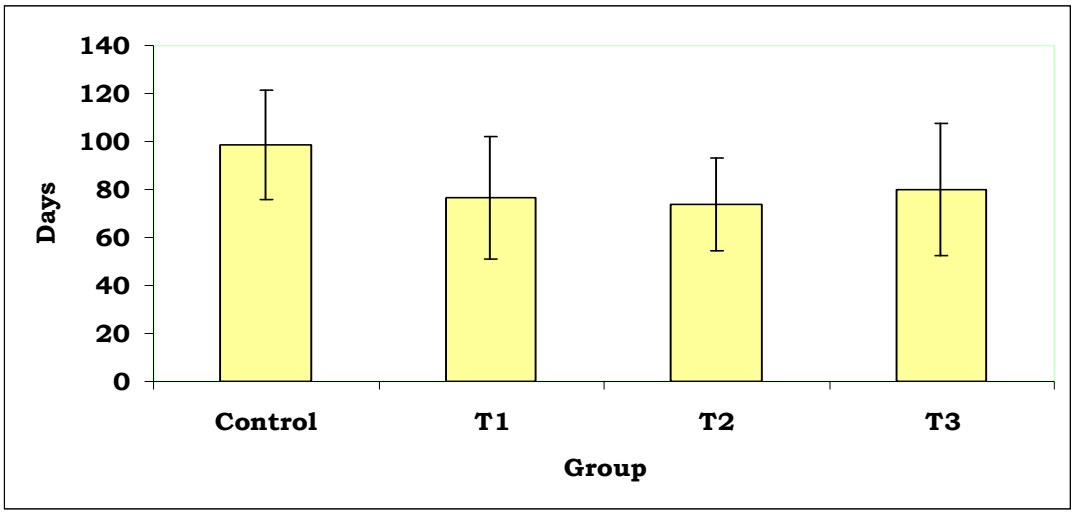
4.6 REPRODUCTION PERFORMANCE

The data on reproduction performance for different groups, evaluated on the basis of days to postpartum heat, conception rate and number of services per conception are presented in table 4.26 and depicted in figures 4.21, 4.22 and 4.23.

4.6.1 Days to Postpartum Oestrus

Mean of first post partum heat in control was 98.80±22.60 days and corresponding values for treatment group T_1, T_2 and T_3 respectively, were 76.70 ±25.57, 73.70 ±19.47 and 80.20 ±27.57. There was no significant ($p \leq 0.05$) difference among control and different treatment groups for days to first postpartum oestrus but cows in T_1, T_2 and T_3 respectively, showed first oestrus around 21, 25 and 19 days prior to the cows in the control group. Estrogenic (Mitra *et al.*, 1999; Pandey *et al.*, 2005) property of shatavari, one of the polyherbal ingredient, which stimulate the ovarian function, improves uterine tonicity thus helps in early uterine involution which consequently results into early initiation of estrus cycle. Additionally, resumption ovarian cyclicity after parturition depends on the nutritional status, body energy reserved and blood glucose level of the animal. As blood glucose is the main source of energy for ovarian function (Rabiee *et al.*, 1997), and influences *in vitro* bovine thecal cell steroidogenesis (Stewart *et al.*, 1995), it may play a major role in achievement of postpartum ovulation. Therefore comparatively early postpartum oestrus can be due to better energy status, as indicated by the lower plasma NEFA concentration (Table 4.16) of cows in treatment groups and estrogenic property of shatavari.

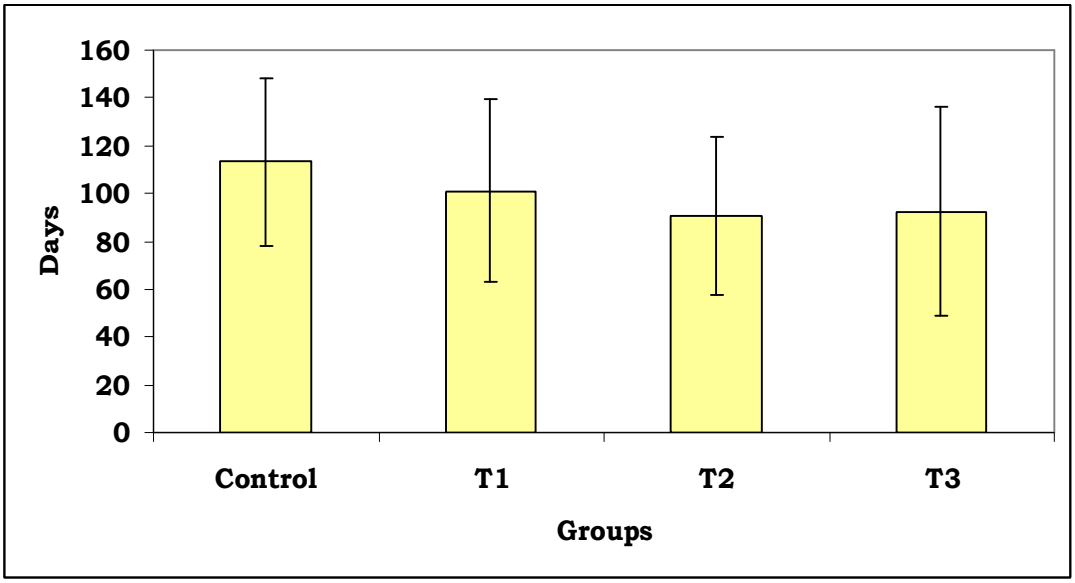
Figure 4.21 Days to postpartum oestrus in different treatment groups



4.6.2 Days to First Service

Average number of days to first service in control group was highest, 113.13 days, followed by 101.25 in T_1, 92.50 in T_3 and 90.70 in T_2. Therefore, In comparison to control, cow in T_1, T_2 and T_3, respectively, received service 11.88, 22.43 and 20.63 days earlier.

Figure 4.22 Days to first service in different treatment groups



4.6.3 Conception Rate

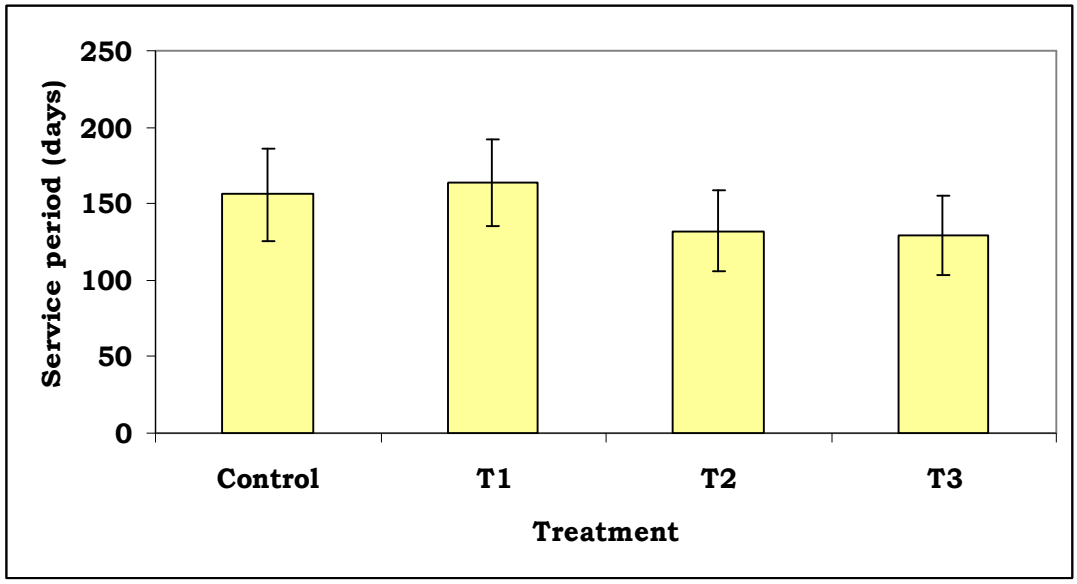
Conception to 1st service was highest, 60% in T_3 followed by 50% in T_2, 30% in T_1 and 40% in control. Further up to 2nd service there was no increase in conception rate in control group, however, among treatments groups, in T_1, T_2 and T_3, respectively, 40, 50 and 80% cows conceived by second service. Maximum, 90% of cows in T_3 conceived up to 3rd service and in T_2 up to 4th service, while maximum conception up to 4th service in control and T_1 was 70 and 80% only. These findings reveals better conception rate in higher supplemented groups (T_2 andT_3) than control and T_1. Canfield and Butler (1990) demonstrated a direct relationship between postpartum energy balance and first ovulation. Negative energy balance results into increased blood urea nitrogen concentration (Dhali, 2001; Campanile *et al.*, 1998). Greater serum or plasma urea nitrogen concentration reduces LH binding to ovarian receptors, leading to decrease in serum progesterone concentration and pregnancy rates (Agarwal and Maurya, 2002; Sharma *et al.* 2006). Barker *et al.* (1998) reported clinical mastitis before first service reduces conception rate and prolonged postpartum heat and service period. Therefore, better conception rate in higher supplemented groups can be attributed to better energy status (Table 4.16) and low or no incidence of clinical mastitis (Table 4.25).

4.6.4 Service Period

Means of service period in control group was 156.29±30.09 days, while corresponding value in T_1, T_2 and T_3 respectively, was 163.37 ±28.15, 131.89 ±26.54 and 129.11±25.28. There was no significant ($p \leq 0.05$) difference in service period among control and different treatment groups; however, service period was apparently highest for T_1, followed by control, T_2 and T_3. In comparison to control, cows in T_2 and T_3 group conceived 24.44 and 27.18 days earlier while in T_1 7.08 days later. This higher service period in control and T_1 might be due to higher incidence of clinical mastitis and negative energy balance in these groups. These

findings were in agreement with those of Huszenicza *et al.* (2005) and Gunay and Gunay, (2008), who also reported that cows suffering with clinical mastitis prior to first postpartum AI and between first postpartum AI and pregnancy diagnosis had significantly extended ($P<0.05$) duration of days open.

Figure 4.23 Average service period in different treatment groups



4.6.5 Number of Services per Conception

The average number of service per conception in control, T_1, T_2 and T_3 respectively, was 2.00, 2.25, 1.88 and 1.55. Higher numbers of service per conception in control and T_1 was due to poor conception rate in these groups. The improvement in higher supplemented groups could be due to anti-oxytotic action of active principles present in shatavari on uterus, which helps in conception (Gaitonde and Jethmalani, 1969). Mitra *et al.*, (1999) also reported that shatavari based herbal formulation did not possess oxytocin like activity which might be useful in uterine hypermotility associated early abortion. Further, Kumar and Singh, (2001) reported that administration of shatavari based herbal formulation increases the TSH, FSH and LH secretion, which help in regular ovulation and thus improved conception rate. Positive energy balance (Table 4.19)

and better micromineral status (Table 4.20.4.21, 4.22 and 4.23) in higher supplemented groups could be attributed for better reproductive performance.

4.7 ECONOMICS OF MILK PRODUCTION

Production economics was calculated for 180 days of lactation (supplementation, residual and post-residual period) by taking into account supplementation cost, veterinary expenditure on treatment of cows over saving on feeding cost due to reduced service period and milk production. Labour cost was not considered as it was constant for all the groups. The data on effect of polyherbal supplementation on economics of milk production are presented in table 4.27 and depicted in figures 4.24, 4.25 and 4.26.

4.7.1 Average Daily Expenditure (Rs./day/cow)

The results reveal that total veterinary expenditure for treatment of cows was lowest in T_3, followed by T_2, control and T_1 in increasing order. Veterinary expenditure in T_2 and T_3 was 20.03 and 45.28% lower than control, however in T_1 it was 7.66% higher than control. This lower veterinary expenditure in T_3 and T_2 was due to lower incidence of clinical mastitis and better reproductive performance of cows in these groups. The cost of polyherbal supplementation (Rs./cow/day) in T_1, T_2 and T_3 was Rs. 9.20, 13.19 16.47, respectively. Extra feeding cost (Rs./cow/day) due to prolonged service period in control, T_1 and T_2 was Rs. 25.23, 31.80 and 2.58, respectively. The highest extra feeding cost due to prolonged service period was observed in T_1 followed by control and T_2. Taking in to account expenditure on supplementation, veterinary treatment and extra feeding due to prolonged service, the total average expenditure (Rs./cow/day) in control, T_1, T_2 and T_3 was Rs. 147.95, 163.94, 137.90 and 138.16, respectively.

Table 4.28 Reproduction performances of cows in different treatment groups

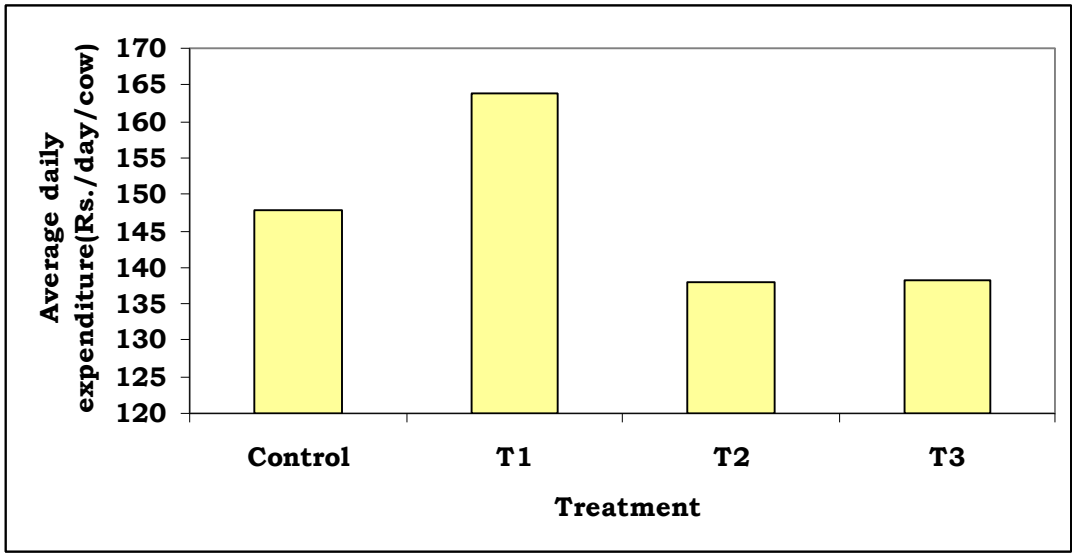
Treatment	Postpartum Oestrus (days)	Days to 1st Service	Conception rate (%)				Service period (days)	Number of services per conception
			Up to 1st Service	Up to 2nd Service	Up to 3rd Service	Up to 4th Service		
Control	98.80±22.60	113.13±35.36	40	40	60	70	156.29±30.09	2.00
T_1	76.70±25.57	101.25±38.21	30	40	70	80	163.37±28.15	2.25
T_2	73.70±19.47	90.70±32.92	50	50	70	90	131.89±26.54	1.88
T_3	80.20±27.57	92.50±43.57	60	80	90	90	129.11±25.28	1.55

Means bearing different superscripts within a row (small) and column (capital) differ significantly (p≤0.05)

Average total expenditure in T_2 and T_3 was 6.79 and 6.61% lower than control; however, in T_1 it was 10.80% higher than control.

Figure 4.24

Average daily expenditure (Rs./cow/day) in different treatment groups



4.7.2 Milk Production Cost (Rs. /Kg)

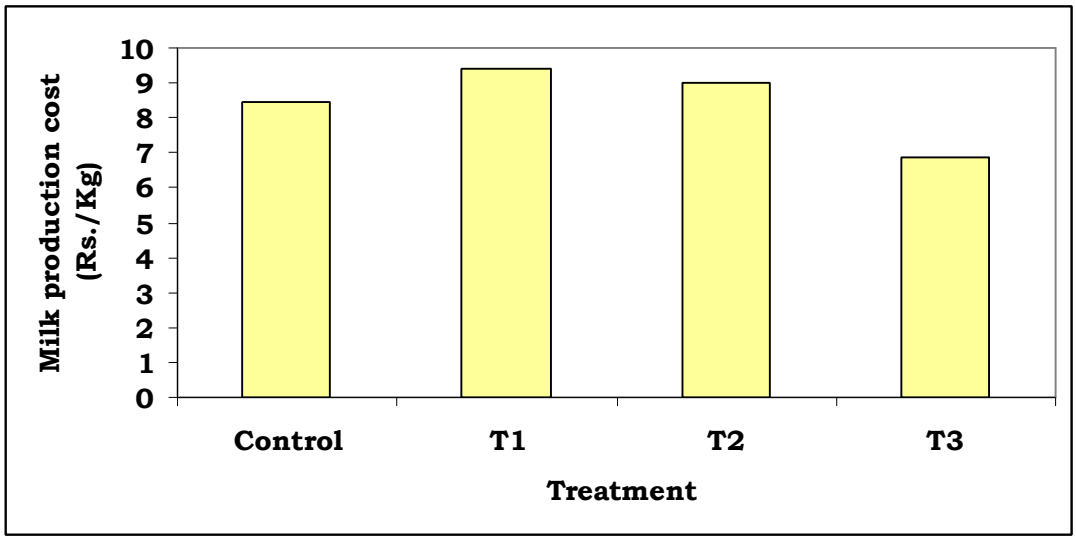
Cost of per Kg milk production in control, T_1, T_2 and T_3 was Rs. 8.46, 9.39, 6.99 and 6.89, respectively. It was highest in T_1 followed by control, T_2 and T_3. In comparison to control cost of per Kg milk production in T_2 and T_3 was 17.37 and 18.55 % lower, whereas, in T_1 it was 10.99% higher than control.

4.7.3 Income over Expenditure (Rs. /day/cow)

Income from sale of milk over expenditure, in control, T_1, T_2 and T_3 was Rs.131.89, 115.26, 177.78 and 182.48, respectively. Income was highest in T_3 followed by T_2, control and T_1. In comparison to control, margin of receipt per cow per day in T_2 and T_3 was Rs. 45.89 and 50.59 higher, however, in T_1 it was lower by Rs.16.63. Percent margin of receipt over control in T_3, T_2 and T_1 was 10.36, 6.15 and -3.34%, respectively.

This can be inferred from these results that polyherbal supplementation @ 200-250 mg/Kg BW is profitable because supplementation at only this dose improved production and reproduction performance and reduces expenditure on veterinary and maintenance cost. This results into higher return and expenditure ratio.

Figure 4.25Cost of milk production (Rs./Kg) in different treatment groups



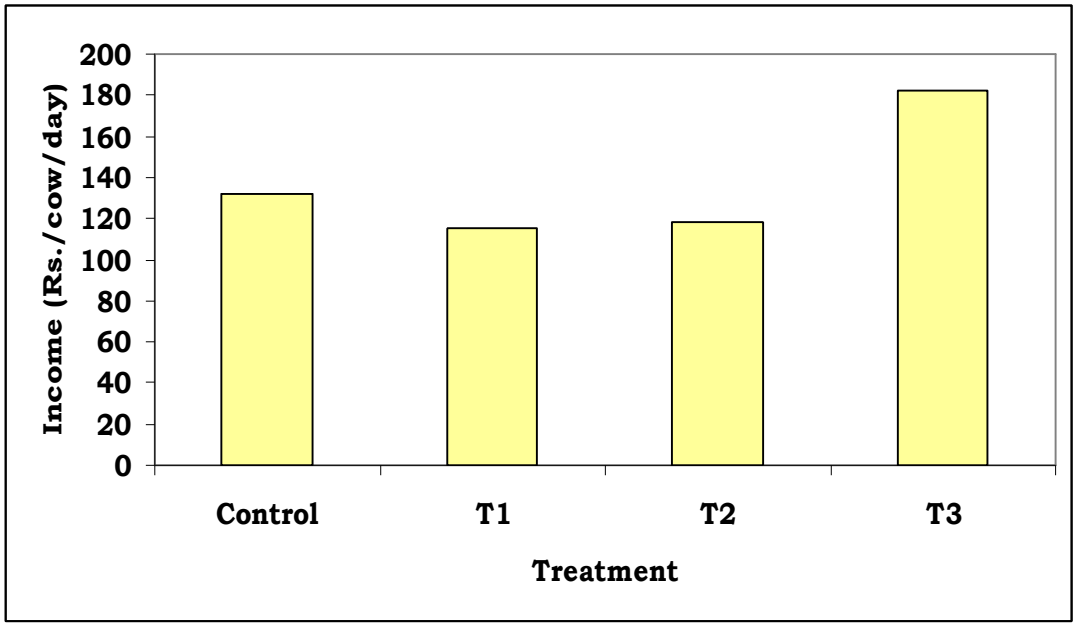


Figure 4.26 Average daily income (Rs./cow/day) in different treatment groups

Table 4.29 Economics of milk production of cows in different treatment groups

Parameter	Treatment			
	Control	T_1	T_2	T_3
Total veterinary expenditure (Rs.)	3445	3709	2744	1845
Total supplementation expenditure (Rs.)	0	11040	15828	20129
Total veterinary and supplementation expenditure (Rs.)	3445	14749	18572	21974
Average veterinary and supplementation expenditure (Rs./cow/day)	2.87	12.29	15.47	18.31
Daily feed cost (Rs./ cow/day)	119.85	119.85	119.85	119.85
Extra feeding cost incurred on account of prolonged service period (Rs./cow/day)	25.23	31.80	2.58	-
Total daily expenditure (Rs./ cow/day)	147.95	163.94	137.90	138.16
Average daily milk yield (Kg/cow)	17.49	17.45	19.73	20.04
Cost per Kg milk production	8.46	9.39	6.99	6.89
Daily return/cow from sale of milk @ Rs.16/Kg	279.84	279.20	315.68	320.64
Income over feed +veterinary+ supplementation +cost of service period saved (Rs/cow/ day)	131.89	115.26	177.78	182.48
Margin of receipt over control (Rs./cow/day)	-	-16.63	45.89	50.59
% Margin of receipt over control		-14.43	25.81	27.72

Chapter Five

Summary and Conclusions

Chapter Five

SUMMARY AND CONCLUSION

Periparturient period is transition period between pregnant non-lactating and non-pregnant lactating physiological state. Therefore, during this period dairy animals in general and high yielding cross breed cows in particular witness several physiological and biochemical changes. These changes exert severe stress on the cow thus making them susceptible to various infectious and non infectious diseases. Therefore, periparturient period is very critical for the health of cows. As parturition is associated with production and reproduction of dairy cows, therefore, high incidence of diseases during peripartum period is a major cause of concern for the dairy farmers. Diseases during peripartum period have severe negative impact on production and reproduction performance of the cows and consequently on economy of dairy farming.

Control of these diseases by treatment using chemotherapeutics has many disadvantages i.e. disease associated production and reproduction losses, heavy expenditure on treatment, drug resistance in animals and human health hazard due to consumption of contaminated animal food.

Therefore, to overcome the problem of periparturient infectious diseases preventive approach is more practical and economical than control by treatment. Therefore, in quest of disease prevention, vaccines have been developed against some specific bacterial and viral diseases but this vaccination approach is not effective against the diseases which are either polymicrobial in origin or microbes having many serotypes or serovars.

As the disease results, when microbes overcome host immune system, therefore, strengthening non specific immunity of host can be a suitable alternate for prevention of diseases to existing conventional

chemotherapeutic approach. Earlier several studies have been conducted using vitamins and minerals having specific role in improving immunity. Herbs like *Withenia somnifera, Ocimum sanctum, Tinospora cordifolia, Emblica officinalis, Nigella sativa, Tribulus terrestris and Asparagus racemosus* are reported to have antioxidant, antimicrobial, immunomodulatory and antiinflammatory property and these herbs are being used in human ayurvedic medicine. Except few studies with individual herbs on production and reproduction performance of large animal no information was available on effect of combination of these herbs on immunity of animals and their effect on diseases incidence. Therefore, keeping in view aforesaid problem this study was planned to develop polyherbal preparation and standardization of its supplementation dose for improvement of immune status of periparturient cows. Present study was conducted on Karan-Fries cows at cattle yard of NDRI, Karnal, Haryana from September 2008 to June 2009. A total of 40 pregnant Karan-Fries cows in 2-6 parity, on the basis of their expected producing ability, were grouped in to 4 homogenous treatment groups of 10 each. Out of these, one group served as control wherein no supplementation was given. Cows in treatment groups T_1, T_2 and T_3, respectively, were subjected to polyherbal supplementation @ of 150,200 and 250 mg/kg BW/day from 60 days prepartum to 60 days postpartum.

Major findings of this study are summarized as follows:

5.1 PRODUCTION PERFORMANCE OF COWS

Results showed that during supplementation (0-2 months), residual (3-4 months) and post residual (5-6 months) period average daily milk yield and FCM yield in T_2 and T_3 were significantly ($p \leq 0.05$) higher than control and T_1. Even after higher milk yield, in comparison to control, no depression was observed in T_2 and T_3 for fat, protein, lactose and SNF %. Due to higher milk production and no depression

fat, protein, lactose and SNF % in T_2 and T_3, fat, protein, lactose, SNF yield were also significantly ($p \leq 0.05$) higher for T_2 and T_3.

5.2 UDDER HEALTH AND MILK QUALITY

Udder health was monitored on the basis of percent infected quarters and average teat score by modified California mastitis test and milk quality on the basis of milk somatic cell count, standard plate count on different fortnightly test day. Results of CMT reveal higher percentage of infected quarters and average teat score for control and T_1 than T_2 and T_3. Accordingly milk SCC (lacs/ml) and SPC (lacs/ml) were also higher for control and T_1 than T_2 and T_3. Therefore, it was inferred that in comparison to control and T_1, cows in T_2 and T_3 had better udder health and consequently better milk quality.

5.3 IMMUNITY STATUS

Immunity status of animals was evaluated on the basis of TLC, DLC, neutrophil phagocytic index, plasma antioxidant activity, NEFA, IgG and microminerals status at different test days during pre and post partum period.

Results reveals no significant difference in TLC among different treatment groups, however, it was higher for T_3, followed by T_2, T_1 and control. Neutrophil concentration among different groups did not vary significantly; however, it was higher for control followed by T_1, T_2 and T_3.

Around parturition at -15, 0 and 15 day of calving, In comparison to control and T_1, neutrophils phagocytic activity was higher for T_2 and T_3.

Antioxidant status, indicated by plasma FRAPS value, showed significant depression in control group towards calving. Antioxidant status at the time of parturition and postpartum period, in T_3 treatment was significantly better than control, T_1 and T_2.

Plasma NEFA concentration is an indicator of energy balance of animals. Significant increase in plasma NEFA concentration towards

calving in all groups indicated negative energy balance but it was highest in control and lowest in T_3. Significantly lower plasma NEFA concentration in T_3 at 0, 15th and 30th day of calving indicated lower feed intake depression and negative energy balance in T_3 group.

Ig G is most fluctuating antibody around calving. Plasma Ig G concentration in T_3 and T_2 at -30th, -15th, 0, and 15th days of calving was significantly higher than control and T_1.

Micro-minerals Cu, Fe, Zn and Mn plays important role in immunity of animals. These minerals serve as cofactors in several antioxidant enzyme systems.

Plasma Cu, Fe, Zn and Mn concentration at different test days during peripartum period in T_2 and T_3 was higher than control and T_1 and this difference was significant at the day of calving.

These results suggest that polyherbal supplementation improved immune status of cows during peripartum period. This could be due to antioxidant property of its ingredients.

5.4 POSTPARTUM DISORDERS

From 60 days prepartum to 60 days postpartum, incidence of clinical mastitis was maximum in control (40%), followed by 30% in T_1 and 20% in T_2. During the experiment none of the cows in T_3 group suffered from clinical mastitis. The incidence of retained placenta in control, T_1, T_2 and T_3 was 10, 10, 20 and 10%, respectively, and incidence of metritis in all groups was 10%. In control, T_1 and T_2 all the cows who suffered with metritis also developed clinical mastitis, whereas in T_3 none of the cows suffered from metritis showed clinical mastitis. These findings suggest that polyherbal supplementation reduced the incidence of post parturient complication through improving immunity and reducing periparturient stress to animals.

5.5 REPRODUCTION PERFORMANCE

Days to return in post partum heat in control group were highest. Cow in T_1, T_2 and T_3 respectively, showed postpartum heat 21,

25 and 19 days earlier to control. Average days to first service in control (113) and T_1 (101) were much higher than T_2 (92) and T_3 (91). In control, T_1, T_2 and T_3 respectively, 30, 10, 50 and 60 % of cows conceived within standard 100 days of service period.

5.6 ECONOMIC OF SUPPLEMENTATION

Economics of supplementation calculated as per day per cow margin of receipt by sale of milk over supplementation and treatment cost indicated highest return in T_3 followed by T_2, control and T_1. In comparison to control, margin of receipt (Rs.) in T_3 and T_2 was higher, however, receipt in T_1 was lower than control. These results suggest that supplementation at the rate of 150 mg/Kg BW was not economical due to higher expenditure on supplementation and treatment with corresponding lower increase in income through sale of milk.

Therefore it can be concluded from this study that:

1. Polyherbal supplementation during periparturient period at the dose rate of 200-250 mg/Kg BW produced beneficial effect on high producing cows.

2. Polyherbal supplementation improved immunity in terms of higher immunoglobulin G concentration, better neutrophil phagocytic activity and plasma micro-minerals profile.

3. Polyherbal supplementation reduced periparturient stress by improving antioxidant status in terms of higher plasma ferric reducing antioxidant power. It also reduced the magnitude of negative energy balance.

4. By improving immune status and reducing peripartum stress, polyherbal supplementation reduced the incidence of sub-

clinical and clinical mastitis and improved udder health, milk yield and milk quality.

5. Supplementation of polyherbal preparation improved reproduction performance as indicated by fewer days to postpartum heat, lower service period and improved conception rate.

6. By improving production and reproduction performance of cows and reducing the expenditure on treatment of diseases polyherbal supplementation improved economics of milk production

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Appendix-1

Price list of polyherbal ingredients

S.N.	Ingredient	Price(Rs./Kg)
1.	Shatavri	104
2.	Ashwagandha	260
3.	Amla	40
4.	Tulsi	40
5.	Giloy	18
6.	Gokhru	48
7.	Klonji	80
Procurement and processing expenses		42.50
Total Price of polyherbal preparation		**137.16**

Appendix-2

Feeding Schedule of cows at NDRI during experimental period

Feed Consumption (cow/day)	Milk Yield (Kg/day)		
	14-17	**17-20**	**20-23**
Green fodder	63.07	50.55	53.23
Dry fodder	0.098	0.052	0.066
Concentrate	6.15	8.59	10.05
Price of fodder (Rs./quintal)			
Green fodder	50		
Dry fodder	200		
Concentrate	1100		
Daily feeding cost (Rs./cow)			
Green fodder	31.54	25.27	26.61
Dry fodder	0.196	0.104	0.132
Concentrate	67.61	94.47	110.65
Total daily feed cost (Rs./Cow)	99.35	119.85	137.39