

**Effect of Shatavari (*Asparagus racemosus*) Root Powder
Supplementation on Performance of Sahiwal Cows**



**THESIS SUBMITTED FOR PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE DEGREE**

OF

MASTER OF VETERINARY SCIENCE

IN

LIVESTOCK PRODUCTION MANAGEMENT

BY

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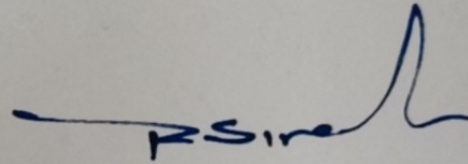
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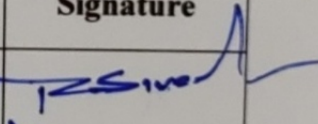
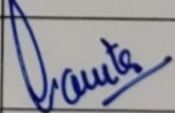
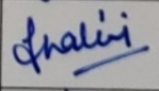
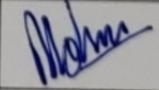
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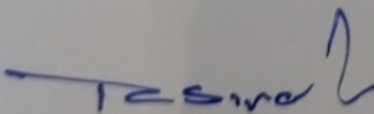
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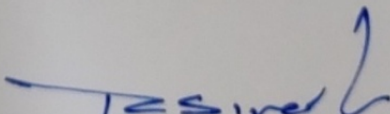
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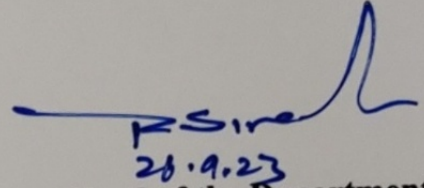
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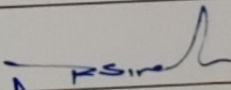
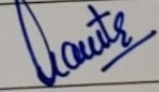
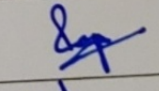
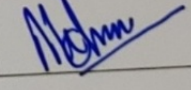
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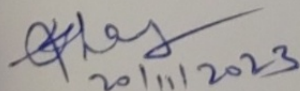
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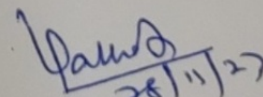
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ABBREVIATIONS

| | | |
|---------------|---|---|
| % | : | Percent |
| & | : | And |
| / | : | Per |
| @ | : | At the rate of |
| < | : | Less than |
| > | : | More than |
| ± | : | Plus Minus |
| °C | : | Degree Centigrade/Celsius |
| μ | : | Mu |
| μl | : | Micro litre |
| μIU/mL | : | Micro litre units/millilitre |
| AOAC | : | Association of official analytical chemists |
| b.wt | : | Body weight |
| BCS | : | Body condition score |
| BHBA | : | Beta hydroxyl butyric acid |
| CF | : | Crude fibre |
| CP | : | Crude protein |
| DLC | : | Differential leucocyte count |
| DM | : | Dry matter |
| e.g. | : | For example |
| EE | : | Ether extract |
| ELISA | : | Enzyme linked immuno-sorbant assay |
| <i>et al.</i> | : | Co-workers/ Et alii /alia |
| etc. | : | etcetera/And so forth |
| Fig. | : | figure |
| Hb | : | Haemoglobin |
| i.e. | : | that is |
| IU | : | International Unit |
| L | : | Liter |
| lit. | : | Litre |
| Ltd. | : | Limited |

| | | |
|--------|---|--------------------------------|
| mEq/L | : | Milliequivalent per litre |
| mg | : | Milligram |
| mg/dl | : | Milligram per deciliter |
| mg/l | : | Milligram per litre |
| mins. | : | Minutes |
| ml | : | Millilitre |
| ng | : | Nano gram |
| ng/mL | : | Nano gram/ml |
| ° | : | Degree |
| OD | : | Optical density |
| PCV | : | Packed cell volume |
| pH | : | concentration of hydrogen ions |
| R | : | Correlation coefficient |
| RBC | : | Red blood cells |
| RPM | : | Round per minute |
| S. No. | : | Serial Number |
| S.E. | : | Standard Error |
| S.E.M. | : | Standard Error Mean |
| SCC | : | Somatic cell count |
| Sec. | : | Second |
| TA | : | Total ash |
| TEC | : | Total erythrocyte count |
| TLC | : | Total leucocyte count |
| WBC | : | White blood cells |
| Wk | : | Week |

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Date **28-11-23**

Manisha Tyagi
(Manisha Tyagi)

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ABSTRACT

The experiment was conducted to evaluate the effect of herbal feed supplement Shatavari root powder in terms of quantitative and qualitative parameters of milk and hemato-biochemical parameters of blood. For this experiments, 18 lactating Sahiwal cows, on the basis of their body weight and initial milk yield were divided into the three treatment groups i.e. T1, T2 and T3 having 6 animals in each. Group T1 acted as control and was provided with only basal diet whereas, T2 group received 30 g Shatavari root powder along with the basal diet and T3 was supplemented with 60 g Shatavari root powder along with the basal feed. The study as a whole was conducted for a period of 90 days. Daily milk yield was also recorded for this whole time. Milk composition of each animal was determined once every fortnight. Blood samples were also collected at fortnight i.e. 0, 15, 30, 45, 60, 75 and 90 days interval.

The effect of herbal feed supplement Shatavari on milk yield was significant ($P < 0.05$) ($6.06^a \pm 0.26$, $6.83^b \pm 0.32$ and $7.35^b \pm 0.17$ in T1, T2 and T3 respectively). Milk composition parameters were also monitored and results revealed no significant ($P > 0.05$) effect of Shatavari root powder on milk fat and protein%. However, milk lactose ($4.47^a \pm 0.05$, $4.47^a \pm 0.05$ and $5.0^c \pm 0.06$ in T1, T2 and T3), SNF ($7.81^a \pm 0.05$, $8.07^b \pm 0.05$ and $8.20^b \pm 0.07$) and total solids ($10.31^a \pm 0.06$, $11.53^b \pm 0.17$ and $12.98^c \pm 0.19$) were found significantly different ($P < 0.05$) from the control group. Physio-chemical parameters of milk i.e. temperature, pH and density were also assessed and found non-significant ($P > 0.05$) in all groups. Milk somatic cell count differed significantly ($P < 0.05$) in T3 compared with control. Effect of SRP on body condition score was found non-significant ($P > 0.05$) in either treatment groups. All hematological parameters monitored like hemoglobin, PCV, DLC, TLC, TEC were found in normal physiological range in all the groups. Cow side test i.e. blood glucose and beta hydroxyl butyric acid (ketone body) were also performed and significant ($P < 0.05$) increase in blood glucose ($34.07^a \pm 0.39$, $39.07^b \pm 0.77$ and $44.38^c \pm 1.21$ in T1, T2 and T3 respectively) was observed in the treatment groups. There was also a significant ($P < 0.05$) reduction in the BHBA after supplementation of herbal feed Shatavari. Biochemical parameters were performed with blood plasma and in these parameters total protein and total albumin were found to differed significantly ($P < 0.05$) in the SRP supplemented groups. Other biochemical parameters like cholesterol, triglycerides, urea and creatinine were also assessed and found to differed significantly ($P < 0.05$) after SRP supplementation. Prolactin hormone was also assessed and found to increase significantly ($P < 0.05$) in T2 and T3 than the control. Cortisol assay was also performed and significant difference was found in the T3 than the control (T1). So, it can be concluded that supplementation of Shatavari in diet improved milk yield and milk composition in terms of SNF, total solids and lactose. Incorporation of SRP in diet significantly improved blood glucose and decreased BHBA. Plasma cholesterol, triglyceride, urea and creatinine were found to be reduced significantly. Results of present study suggests that feeding of SRP @ 60 g/animal/day is beneficial for improving milk yield even in mid phase of lactation, milk composition, glucose concentration and other biochemical constituents in lactating Sahiwal cows.



Introduction



CHAPTER-1

INTRODUCTION

India is a developing country and a land of villages where more than 70% of the human population still depends upon the agriculture and livestock sector for their livelihood and nutritional security. Developing countries like India are facing challenges due to continuously increment in human population day by day and consequent need of rapid increase in the productivity of agriculture and its allied sectors. The demand for protein of animal origin is increasing rapidly, to meet the nutritional requirement of growing populations. Livestock sector plays a vital role in the rural economy, as providing family income and generating gainful employment in the rural sector throughout the country.

India is the highest milk producer in the world, contributing 23% of world milk production, Uttar Pradesh stands second in milk production with 14.93% share while Rajasthan is the leading state in terms of milk production and contributing 15.05% of country's milk (BAHS, 2022). Various measures have been initiated by the Government to enhance the productivity of livestock, which has resulted in significant increase in milk production over the past decade. India has large cattle population (192.49 Million). As per BAHS, 2022, total milk production in the country is 221.06 million tones with a growth rate of 5.29% and the per-capita availability of milk is 444 grams per day. The milk production per animal in India is very low (Exotic cattle- 9.15 lit/day, Cross bred 7.22 lit/day, Indigenous cattle 3.34 lit/day). This lower productivity can be attributed to many factors including the genetic and environmental factors such as non-availability of good quality feed resources, poor husbandry management practices and the small-scale dairy production units (Sharma, 2003). Although the average productivity of indigenous cattle is 3.34 lit/day, yet we have Sahiwal, one of the best indigenous (zebu cattle) dairy breed.

Various drugs, herbal preparations, hormones, mineral supplements and feed additives have been evaluated with variable results to restore the animal's productivity and to optimize the milk production in individual animals for better profits (Ramesh et al., 2000). Galactogogues are medications or herbal supplements believed to assist in the initiation, maintenance, or augmentation of milk production

(Gabay, 2002). The herbal galactogogues are known to have favourable effect on milk production (Bharti et al., 2012). These herbal supplements antagonize the dopamine receptors and augment prolactin secretion (Gabay, 2002). Galactogogues triggers the alveolar tissue and enhance the secretory activity and thereby restore and regulate milk yield (Ravikumar and Bhagwat, 2008). A number of herbal plants have been accentuated in Ayurveda, which contain large number of chemically active principles like alkaloids which have galactogenic properties and can be used as herbal medicine for improving milk production and milk let down efficiency in dairy animals viz. Ashwagandha (*Withania somnifera*), Jivanti (*Leptadenia reticulata*), Chandasoor (*Lipidum sativum*), Kanchari (*Alternanthera sassilia*), Methi (*Trigonella foenumgraecum*) and Shatavari (*Asparagus racemosus*), and many more are known for galactogogue effect (ICMR, 1985).

Shatavari (*Asparagus racemosus*) is most frequently used herbal galactagogue in traditional medicine due to the presence of steroidal saponins and sapogenins in various parts of the plant (Kapoor, 2001; Krishna et al., 2005). Shatavari is a creeper grown in all tropical and sub- tropical countries including in India since prehistoric times. As the name indicates “Shat” means hundred and “Vari” means curer so as a whole it is considered as curer of hundred diseases. This plant is locally called shatavar in Hindi and in central Himalayan region known as satmuli *i.e.* Shata means hundred and muli means roots (Saini and Choudhary, 2018). In Sanskrit this plant is called as Shatavari which means ‘able to have one hundred husbands’ and in Ayurveda this amazing herb is known as the “Queen of herbs”. Shatavari is also rich in active constituents such as steroidal glycosides, saponins, polyphenols, flavonoids (Sharma, 1981) and alkaloids (Wiboonpun et al., 2004)

Around the world 300 species of genus *Asparagus* are common and out of them 22 species are found in India. Out of several species of *Asparagus* grown in India *A. racemosus* is most commonly used in indigenous medicine (Simon, 1997). The leaves are identical to pine needles, small and uniform and the flowers are white with small spikes. It belongs to Liliaceae family found throughout India with 1 to 2 m in height (Alok et al., 2013).

The best Indian dairy breed Sahiwal have well developed udder and naval flap. It is heat tolerant, disease resistant, bloat resistant, and well known for ease of calving and having a good temperament. Average milk yield per lactation of Sahiwal

cattle is around 2325 kg and average milk fat is 4.9%. Average calving interval is around 475 days (NBAGR). To achieve the optimum productivity is the continuous endeavour in dairy management. As a part of these endeavour various herbal feed supplements, galactogogues, growth promoters, prebiotics & probiotics, antibiotics, hormones, mineral supplements and feed additives have been used in order to improve productivity of different dairy animals. Number of studies on Shatavari in livestock (crossbred cattle), (Ramesh et al., 2000 , Thakur et al., 2006, Bhatt et al., 2009), buffalo (Dangi., 2011, Kumar et al., 2011, Patel et al., 2013 , lactating goats (Mirzaei et al., 2012) have reported a positive effect on milk yield.

Although, there is enough review available about positive effect of Shatavari on milk yield but studies in specific phase of lactation are very few and very little literature is available about effect of Shatavari supplementation in particular phase of lactation.

In the view of these facts, the present study was planned to determine the effect of Shatavari root powder as a galactogogue on performance of lactating Sahiwal cows in mid phase of lactation with following objectives:

Objectives

- 1) To study the effect of Shatavari root powder supplementation on milk yield and milk composition of lactating Sahiwal cows.
- 2) To study the effect of Shatavari root powder supplementation on haemato-biochemical parameters.



Review

of

Literature

Growth and milk production in dairy animals is influenced by genetic and non-genetic factors. The non-genetic factor includes environmental conditions, stage of lactation, parity, age of animals, nutritional status and management of animals. On one hand India has large livestock population (536.76 Million) and on the other the country is facing decline in feed resources for livestock. Feed and fodder availability in India is one of the considerable issues in livestock production. Now a days, where the available fodder can meet the demand of only 56% of the total livestock (DAHD, 2014). There is currently a net deficiency of 35.6% green fodder, 10.95% dry fodder and 44.0% concentrate feed materials in the country (IGFRI Vision, 2050). In such situation there is need to explore other alternatives as Various herbal feed supplements, growth promoters, prebiotics & probiotics, antibiotics, hormones, mineral supplements and feed additives in order to improve productivity of animals as well as to optimize growth, health, milk production and milk composition parameters.

Majority of our livestock owners belongs under poverty line, they cannot afford to purchase costly feed supplements available in market. For them herbal formulations could be better alternatives. This chapter includes some studies about different herbal alternatives of galactogogues in dairy animals.

1. Effect of Shatavari root powder on milk yield and milk composition

Ramesh et al. (2000) studied the effect of supplementation of Galactin (Shatavari based polyherbal galactogogue) @ 50 g/day/animal in lactating crossbred cows and reported that significant improvement in milk production than control group.

Thakur et al. (2006) studied effect of 10 herbal feed supplementation on the performance of lactating cows. Result showed that after herbal feed supplement milk and fat corrected milk (FCM) yield improved ($P < 0.01$) without affecting its composition. It was concluded that dietary supplementation of a commercial feed additive @ 10 g/day to lactating crossbred cow increased the milk yield.

Mishra et al. (2008) evaluated the dose of Shatavari as feed supplement for dairy animals @ 50 g/day/animal and 100 g/day/animal irrespective of body weight. It was concluded that supplemented group have significantly ($P < 0.05$) increase in milk production.

Tanwar et al. (2008) studied the effect of Shatavari root powder supplementation on milk production in lactating dairy animals (10 buffaloes and 10 cross bred cows @ 50 gm in concentrate once in a day for a period of 60 days. They find out that overall milk production were increased 1.06 ± 0.17 kg (11.47%) daily and average milk production in buffaloes and cows were increased 0.8 ± 0.34 kg (9.0%), 1.32 ± 0.15 kg (12.72%) respectively as compared to their previous production.

Santosh (2009) evaluated the effect of supplementation of Shatawari root powder @ 100mg/kg live body weight at pre-partum and @ 200 mg/kg live body weight at post-partum significantly ($P < 0.05$) increased milk production by 20% in pre-partum while 10.11% in post-partum stage.

Bhatt et al. (2009) evaluated the effect of feeding two herbal preparations (Ruchamax & Payapro) on the milk yield in lactating crossbred cows. Fifteen lactating cows were taken for the experiment after 3 days of calving to their full lactation. The cows were randomly divided into three uniform groups of 5 cows in each. The animals in group I acted as control. The animals in second and third group were given either Ruchamax @ 30 g per day or Payapro @ 4 tablets per day, respectively for 15 consecutive days in a month for 3 months, commencing 3 days after calving in addition to the usual feed/fodders and were termed as Ruchamax supplemented and Payapro supplemented animals. Significant ($P < 0.05$) differences were observed in milk yield of cows fed herbal preparations as compared to control.

Divya (2010) evaluated the effect of Shatavari root powder on the milk composition. Experiment was conducted on 10 lactating cows (two cows in each group) with similar age group named as T1 (0 gm), T2 (100gm), T3 (150 gm), T4 (200 gm) and T5 (250 gm) respectively. During the experiment, the effect of SRP containing ration on fat, protein, lactose and total solid content was 3.5, 3.63, 3.69, 3.97 and 3.66 % fat, 3.41, 3.58, 3.66, 3.77 and 3.67 % protein, 4.87, 4.78, 4.76, 4.75 and 4.78 % lactose and 12.46, 12.63, 12.78, 13.08 and 12.83 % total solid for T1, T2, T3, T4 and T5 respectively.

Gupta and Garg (2009) evaluated the mode of administration of Shatavari says that Ksheerpaka (milk decoction) of Shatavari is more potent as galactagogue than any other form of dosage. In view of this milk decoction of Shatavari can stimulate more milk production in lactating females, experiments were performed to determine the potentiality of it over aqueous decoction of Shatavari as galactagogue in rats. Female rats that received oral doses of milk decoction of Shatavari (100mg/kg BW) during their first lactation produced about 27% more milk than controls ($P < 0.05$).

Sharma (2010) reported that polyherbal supplementation containing Shatavari @ 200mg /kg BW, significantly increased the milk yield by 12.24, 15.01 and 10.50 % during supplementation, residual and post residual periods, respectively. Whereas, milk yield in dose of polyherbal supplementation @ 150 mg/kg BW, during supplementation, residual and post residual periods, respectively, was found 8.67, 11.46 and 10.50 % higher than control.

Dangi (2011) reported that herbal feed supplementation @ 100 mg/kg/day and 150 mg/kg/day during pre-partum(60 days) while @ 200 mg/kg/day and 300 mg/kg/day during post-partum stage(90 days) in 15 murrah buffaloes (divided in 3 groups) . The result indicated that milk and FCM yield during supplementation period was significantly ($P < 0.05$) higher in treatment group than control group.

Kumar et al. (2011) studied on twenty eight experimental lactating Murrah buffaloes were selected and divided into four groups on the basis of their body weight, milk yield, parity and stage of lactation. The trial was conducted for five months. The different dietary groups were T1: Normal feed (control), T2: feed with 50 g Chandrasoor seed powder (CSP), T3: Feed plus 100 g CSP and T4: Feed plus 150 g CSP. Total milk yields for the period of 5 months in the buffaloes of the T1, T2, T3 and T4 groups were 1178.3, 1232.4, 1240.8 and 1257.9 litres, respectively.

Santosh (2011) evaluated the effect of Shatavari root powder supplementation in lactating cross bred cows in pre-partum up to 60 days @ 100 mg per kg live body weight to continue postpartum period up to 90 days @ 200 mg per kg live body weight on milk yield and composition. The results indicated that supplementation of Shatavari root powder improved milk production as well as milk composition and increased net return per litre milk significantly in crossbred cows.

Singh et al. (2012) investigated the effect of Shatavari feeding in selected 10 dry and pregnant murrah buffaloes on hormones, metabolites, milk yield, and plasma cholesterol levels. The treatment groups of buffaloes were fed with Shatavari root powder @ 150 g/day/animal during pre-partum and @ 300 g/day/animal during the postpartum period. The buffaloes under treatment group produced more milk (@0.526 kg/animal/day) suggesting thereby that Shatavari is galactopoietics in nature.

Mirzaei et al. (2012) carried out the effect of dried mixture of five herbal plants as natural additives on milk yield and composition of cross bred dairy goats. Thirty lactating goats after two weeks of kidding were divided into 3 groups (n= 10) using complete random block design to evaluate the effect of herbal mixture supplement on the productivity of lactating goats. T2 and T3 were supplemented with polyherbal combination @ 125 and 250 mg/kg BW respectively. Goats without polyherbal combination served as control group T1. Milk yield, 4% FCM, milk protein, fat, total solids were significantly higher for animals fed T2, followed by T3 and then T1.

Patel et al. (2013) worked out on 22 lactating Surti buffaloes were randomly allotted to treatment and control group (11 buffaloes in each group). Two polyherbal galactogogue biscuits (19 g each) per day were fed for initial 10 days of the month over a period of three months to buffaloes in treatment group. The daily milk yield of buffaloes was recorded. A significant higher ($p<0.05$) total milk yield (14.24 %) of treatment group was recorded.

Kumar et al. (2014) also reported that cows supplemented with Shatavari root powder @ 100 mg/kg body weight to advance pregnant cows were produces significantly higher milk yield ($P<0.01$) than control group.

Sukanya et al. (2014) observed that supplementation of milkplus a Shatavari based herbal prepration enhanced the milk yield from 8.26 to 10.11 litres/day in experimental animals than control.

Galbat et al. (2014) evaluated the effect of dried mixture from seeds of four herbal plants as natural additives on milk yield and its constituents in Egyptian dairy goats. The herbal plants are Cumin (*Cuminum cyminum*), *Trigonella foenumgraecum* seeds, *Carum carvi* and *Nigella sativa* in equal ratio. Eight lactating goats after two weeks of kidding were divided into 2 groups (four animals each) to evaluate the effect

of herbal mixture supplement on the productivity of lactating goats, the 1st group (G1) was supplemented with polyherbal combination at the rate of 250 mg/kg body weight, the 2nd group (G2) was served as Control. Animals in each group were fed on 40% concentrate feed mixture and green fodder ad libitum. Individually, milk, serum and blood samples were collected weekly along the experimental period (12 weeks). Results obtained indicated that. Milk composition showed milk protein, TS and SNF contents were higher ($p > 0.05$) in animals fed experimental additives than control. Milk yield in the present study was slightly higher ($p < 0.05$) in supplemented group compared to control group, animals fed supplemented rations had higher ($p < 0.05$) glucose concentrations than control. Most of blood parameters are within the normal range that means the addition of such additive had no adverse effect on blood components.

Divya et al. (2015) had observed that supplementation of Shatavari root powder in lactating cows leads to increase in milk yield significantly without altering milk quality and natural attributes.

Jain and Bais (2015) conducted study on 16 lactating Kankrej cows having homogenous parity, milk yield and body weight to investigate the effect of Jiwanti (*Leptadenia reticulata*) supplementation on Fat % and fat yield of milk produced by Kankrej cows. Cows were grouped in to 4 homogenous treatment groups of 4 each. Cows in treatment groups T1, T2 and T3 respectively, were subjected to Jiwanti supplementation @ 50, 100 and 150 gm. per day. During the supplementation period the Fat percentage was found to be non-significant ($p \leq 0.05$) in all the treatment groups however during post supplementation fat yield was highest in T3 (30.25% higher than control) followed by T2, T1 and then control but the differences were non-significant.

Soni et al. (2016) conducted study to investigate the effect of Shatavari supplementation during postpartum period on production performance of 16 lactating Kankrej cows @ 50, 100 and 150 g per day in T1, T2 and T3 treatment groups, respectively, the fat% was found significantly higher ($P \leq 0.01$) in T3 group than the control and T1 group however, there was no significant difference between control, T1 and T2 group. The fat yield was significantly higher in T1, T2, T3 group than control but there was no significant difference found between the different treatment groups.

Das et al. (2017) study was conducted to evaluate the effect of feeding 5 herbal preparations on the milk yield and composition of crossbred cows. Thirty five lactating cows of first-third lactation were selected for the experiment. The cows were divided into 7 groups with 5 cows in each group on the basis of average milk yield (8.70 ± 0.08 kg/d). The experiment was conducted for 90 days, partitioned into pre-treatment period (15 days), treatment period (60 days) and post treatment observation period (15 days). The cows in group C₀ were acted as control. Cows in groups T₁ and T₂ were given either Restobal @ 50ml twice daily for 5 days/month or Ruchamax @ 15 g/ day for 8 days/month, respectively for 60 days treatment period. The cows of groups T₃, T₄ and T₆ were supplemented either Payaboon @ 500 g/ton, Payaboon @ 1 kg/ton, Oprium at dose rate of 500 g/ton/ day, respectively for 60 days time period. Cows of T₅ were fed Payapro@ 4 bolus/day for 15 consecutive days commencing after 15 days pre-treatment period. T₅ group revealed comparatively higher total milk yield than the control and other groups. Average milk yield (kg/d) during the experimental period was slightly higher in T₅ followed by T₂ and T₆. T₅ group showed lowest trend of reduction in milk yield during treatment period. Post supplementation milk yield (kg/d) was also found higher in T₅. Milk protein and fat (%) were higher in T₁ and T₃ than the control and other treatment groups. Total solid and SNF (%) showed higher trend in T₁. At 15 day of supplementation, milk yield was found to increase in all the treatment groups compared to control (8.38) and it was highest in T₅ (9.69). The milk yield decreased gradually thereafter but the rate of reduction was comparatively lower in T₂, T₅ and T₆ than the control. Milk components revealed significant variations among different weeks in different groups. Hence it can be concluded that herbal preparations can increase the productivity in lactating dairy cows without deleterious effect on general health of animal.

Patel et al. (2017) evaluated the efficacy of herbal galactagogue mixture containing *Asparagous racemosous* (Shatavari), *Leptadenia reticulata* (Jivanti) and *Trigonella foenum-graecum* (Fenugreek) on production performance of lactating Kankrej cows. 24 lactating Kankrej cows were divided into four treatment groups. The T₁ group was fed with basal diet without herbal mixture supplementation (control); groups T₂, T₃ and T₄ were fed with basal diet + 30, 60 and 90 g/cow/day herbal mixture supplementation, respectively. The dry matter intake was found comparable among the treatment groups. Average daily milk yield and 4% fat

corrected milk yield were significantly ($P < 0.05$) higher in herbal galactagogue supplemented groups as compared to the control group. Supplementation of Shatavari, Jivanti and Fenugreek in equal proportion at the dose rate of 60 g/cow/day resulted in significant increase in milk yield lactating Kankrej cows.

Kumawat et al. (2017) studied the effect of *Asparagus racemosus* supplementation on body condition score, milk production and composition was studied in crossbred cattle. 30 crossbred cattle in mid-lactation were randomly and equally distributed into two groups as control non-supplemented, CS group and *A. racemosus* supplemented, AS group. AS group cows were fed *A. racemosus* root powder @ 125 mg/kg body weight per day for a period of 60 days. Body condition score was increased ($p < 0.01$) from 3.75 to 4 in AS group during 60 days. 4% fat corrected milk yield (FCM) yield (kg/day) was higher ($p < 0.01$) and increased by 16.94% in AS group over CS group. Milk fat and total solid contents were increased ($p < 0.01$) in AS group as compared to CS group, whereas other composition values statistically indifferent from the control. Hence, it was concluded that *A. racemosus* root powder can be supplemented for the improvement of animal performance.

Jingar et al. (2018) conducted a farm trial to evaluate the effect of Shatavari root powder @ 50 g/day/animal on milk production in 20 lactating buffaloes for a period of six months. The milk production was increased by 9.67% daily as compared to their previous production. Increase in the income by feeding Shatavari came to Rs. 12.00/day/animal and B:C ratio was found 1:2.28. From the above results it was concluded that Shatavari is effective for increase in milk production and beneficial for economic milk production.

Kumar and Kumar (2018) evaluated the effect of feeding three different herbal galactagogue preparations like Galactin Vet, Payapro and Gomilk on the milk yield and its composition in crossbred cows. Twenty lactating crossbred cows were taken for the experiment and were randomly divided into four groups of five cows each i.e. T0 (control group) without any galactagogue. Cows in T1 group supplemented with Galactin Vet @ 4 boli/day, Cows of T2 group supplemented with Payapro @ 4 boli/day orally while T3 cows supplemented with Gomilk @ 10 tab bid orally per day for a period of two months. Significant ($P \leq 0.01$) difference was seen in the milk yield of cows fed with herbal galactagogue as compared to the control. Highest average milk yield of 7.76 ± 0.16 litres per day (4% FCM) was seen in Payapro

supplemented cows followed by 7.54 ± 0.23 litres/day and 7.01 ± 0.15 litres/day in the case of cows supplemented with Galactin Vet and Gomilk respectively. Average milk yield was observed 5.95 ± 0.19 litres/day in case of control group. Payapro supplemented cows showed 19.64% increase in milk yield where as in the case Galactin and Gomilk supplemented cows showed 14.01% and 10.83% increase in milk yield respectively. In T0, T1, T2 and T3 group cows the increase in fat percentage was observed to be $4.01 \pm 0.04\%$, $4.26 \pm 0.03\%$, $4.34 \pm 0.03\%$ and $4.46 \pm 0.03\%$ respectively during the trial period where as mean SNF percentage of T0, T1, T2 and T3 group of cows increased to 7.32 ± 0.03 , 7.46 ± 0.04 , 7.71 ± 0.05 and 7.84 ± 0.04 respectively. After supplementation of galactogouges, mean fat percentage increase of T1, T2 and T3 groups of cows increased by 3.40%, 5.60% and 9.31% respectively where as mean SNF increased by 3.32%, 3.49% and 2.75% respectively.

Saini et al. (2018) conducted study to evaluate the effect of herbal feed supplement Shatavari (*Asparagus racemosus*) in terms of qualitative and quantitative parameters of milk, in crossbred cows. The cows were divided in four groups viz. G1 (control), in which the animals did not received supplements, whereas the animals of group 2 (G2), group 3 (G3) and group 4 (G4) were supplemented with Shatavari root powder @ 40 gm, 80 gm and 120 gm respectively. The study was executed for a period of 60 days. Milk composition parameters were determined once every fortnight. Cows fed Shatavari root powder produce significantly ($P \leq 0.01$) more milk and milk fat per cent, milk fat yield, milk protein per cent, milk protein yield, milk lactose per cent, milk lactose yield, milk solid not fat per cent, milk solid not fat yield, milk total solid per cent and milk total solid yield content than control cows.

Birhanu and Rita (2019) studied the effect of Shatavari root powder on milk yield and composition. In control phase animal diet normal ration, whereas in the treatment phase, 120 gram of *Asparagus racemosus* root powder in addition to its normal diet per buffalo per day. The result showed that the diet supplemented with Shatavari root powder had significant effect on milk yield at 5% (0.0196), protein at 1% (0.0113) and fat at 1% (0.0007).

Panchasara et al. (2019) evaluated the effect of feeding herbal galactagogue preparation (Sanjivani biokseera) on the milk yield and milk constituents in lactating Kankrej cows. Thirty-two lactating Kankrej cows were taken for the experiment from 3 days after calving up to 52 days postpartum. The cows were randomly allotted into

two groups of 16 cows each according to initial milk yield and milk composition. Group I acted as control and Group II animals were provided with Sanjivani biokseera (Naturewell Industries) @ 60 g per day for the duration of one month, commencing 3 days after calving. A understandable difference was observed in milk yield from day 8 onward of experiment between groups with significant ($p < 0.05$) higher values in herbal galactagogue group as compared to control, but no such distinct effect on milk constituents was observed on day 52 when analyzed. The use of herbal galactagogue significantly ($p < 0.05$) increased the overall average of 52 days milk production, which was 9.34 ± 0.21 lit/day in supplemented as compared to 7.75 ± 0.26 lit/day in control animals.

Gautam et al. (2019) evaluated the effect of polyherbal supplementation on milk yield and milk quality parameters of crossbred cows. The result shows that supplementation of polyherbal mixture significantly increased ($P < 0.05$) the average milk yield in treatment group (8.70 kg/day) as compared to control group (7.38 kg/day).

Muwal et al. (2020) assessed the effect of supplementation of Shatavari (*Asparagus racemosus*) root powder @ 50 g/head/day for 90 days on twenty lactating Sahiwal crossbred (Holstein Friesian x Sahiwal) cows in early stage of lactation. It was observed that the average fortnightly milk production was significantly ($P < 0.05$) higher in treatment group than control (10.87 ± 0.41 kg/d Vs. 9.88 ± 0.15 kg/d).

Bhinda et al. (2020) conducted study to evaluate the effect of Shatavari supplementation on milk production and its composition in crossbred cows. Fifteen lactating crossbred cows were divided into three groups; T₁ (control), T₂ (150 mg/kg body weight) and T₃ (200 mg/kg body weight) of five animals for 90 days duration. The milk yield and fat corrected milk yield were found to be significantly higher ($P < 0.05$) in Shatavari supplemented groups than control group. Average milk fat%, SNF%, total solid% and protein% were significantly ($P < 0.01$) higher in supplemented groups as compared to control group.

Chavan et al. (2021) assessed the effect of Shatavari root powder on milk quality in 20 lactating crossbred cows, were randomly divided into four homogenous treatment groups, five animals each. Experimental groups T₂, T₃, and T₄ were fed a basal diet as per BIS with 40 gm, 80 gm, and 120 gm/cow/day Shatavari root powder

(SRP), respectively. It was observed that SRP supplemented cows produce significantly higher ($P<0.05$) milk fat %, fat yield (kg), solid not fat %, solid not fat yield (kg), protein %, protein yield (kg), lactose %, lactose yield (kg), total solid %, milk total solid yield over control (T1) group.

Bhargav et al. (2021) selected twelve multiparous lactating cows at early stage of lactation and randomly allotted to two equal groups ($n=6$) on the basis of milk yield. Cows in groups T1 was supplemented with cumin seed @ 50 g/d/head for 90 days. Udder health was monitored by modified california mastitis test, measuring milk pH and SCC (somatic cell count) at fortnightly intervals. The average milk yield (kg) of animals was statically non-significant ($P>0.05$) but numerically 20.64% higher milk yield was observed in cumin seed supplemented group than control group. There was no effect of cumin supplementation on composition (Fat, TS, SNF, Lactose and Protein) of milk in dairy cows. Overall mean milk pH and somatic cell count (SCC) was numerically lower in treatment group. From the results it can be concluded that cumin seed (*Cuminum cyminum*) supplementation improves milk yield by 20.64%. However, no effect was observed on composition of milk in dairy cows.

Khera et al. (2022) the present investigation was done to study the effect of Shatavari (*Asparagus racemosus*) on the performance of lactating crossbred cows. Twelve crossbred cows were divided into control (T0) and treatment (T1) having 6 cows in each group. Cows having same stage of lactation and parity were selected. Cows of both the groups were fed standard diet (NRC, 2001) with supplementation of Satomul @ 100 gm daily in the treatment group for 6 weeks. The parameters studied were body condition score (BCS), daily milk yield (DMY), milk composition. The overall average BCS of T0 and T1 groups was 3.41 ± 0.05 and 3.51 ± 0.07 , respectively and they differed significantly ($p<0.05$). The overall average fat, solid-not-fat (SNF), protein, lactose and total solids (TS) was 4.32 ± 0.11 and 4.99 ± 0.09 ; 8.89 ± 0.06 and 8.93 ± 0.07 ; 3.41 ± 0.03 and 3.54 ± 0.05 ; 4.67 ± 0.04 and 4.68 ± 0.05 and 13.21 ± 0.14 and 13.92 ± 0.12 percent in T0 and T1 groups, respectively. The treatment with Shatavari had significant effect on milk fat ($p<0.05$). The variation of acidity of the milk was non-significant ($p>0.05$) between T0 ($0.140\pm 0.004\%$) and T1 ($0.147\pm 0.003\%$).

2. Effect on haemato-biochemical and hormone parameters

Shelukar (2001) also reported that supplementation of Shatavari and Ashwagandha (*Withania somnifera*) and probiotics significantly increases haemoglobin and blood glucose in supplemented group over control group.

Dadkhah and Yeganehzad (2011) reported that supplementation of galactogogue herbal mixture in dairy cows had higher levels of prolactin hormone and insulin which lead to increased milk production.

Singh et al. (2012) investigated the effect of *Asparagus racemosus* feeding in selected 10 dry and pregnant Murrah buffaloes on hormones, metabolites and plasma cholesterol levels. The treatment groups of buffaloes were fed with Shatavari root powder @ 150 g/day/animal during prepartum and @ 300 g/day/animal during the postpartum period. Shatavari root powder supplementation leads to significantly ($P<0.01$) increased plasma prolactin, cortisol ($P<0.01$), and milk fat cholesterol ($P<0.05$) without affecting total cholesterol, HDL, LDL, glucose, and NEFA concentrations.

3. Somatic cell count

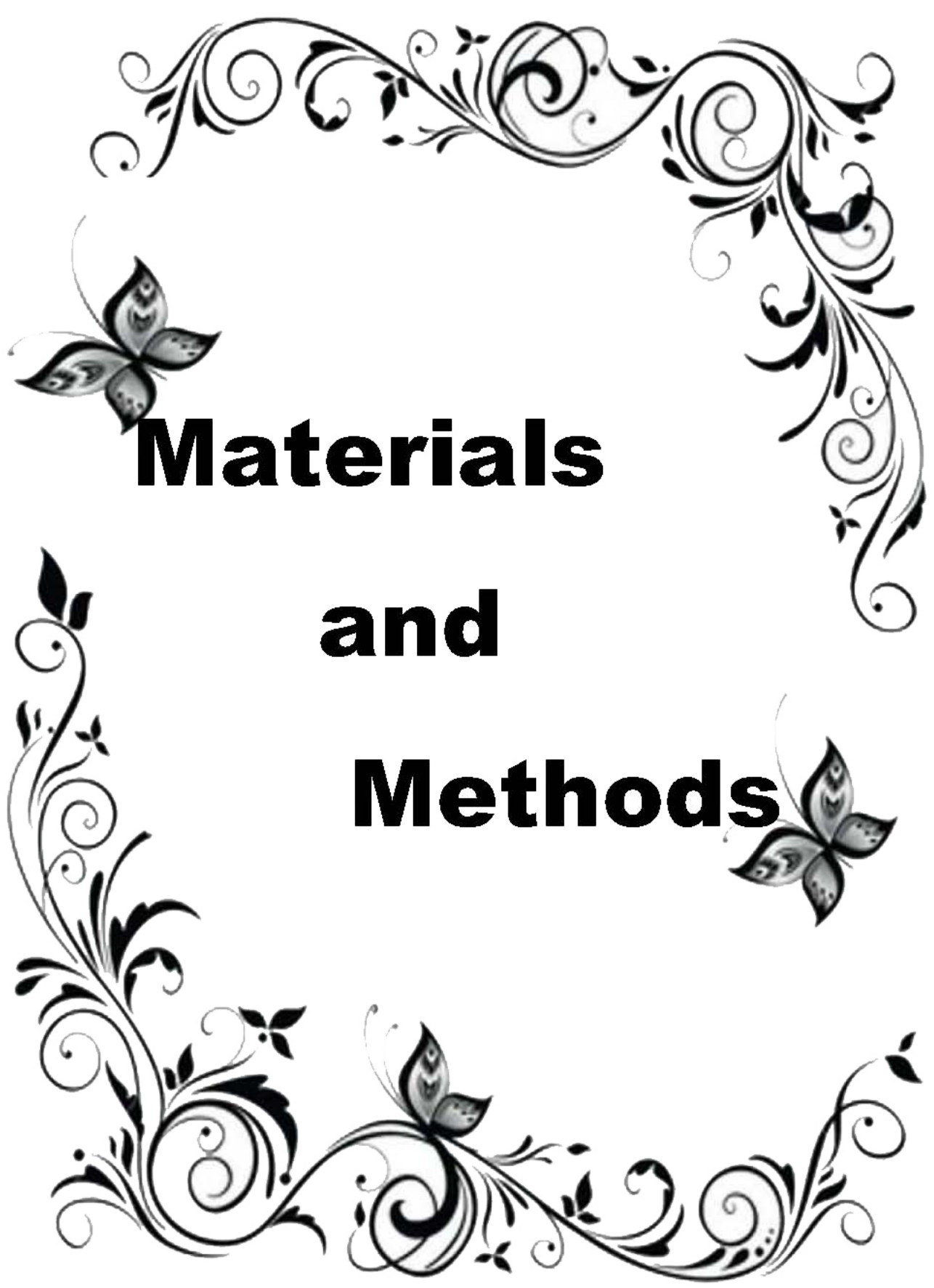
Dangi (2011) reported that herbal feed supplementation @ 100 mg/kg/day and 150 mg/kg/day during pre-partum while @ 200 mg/kg/day and 300 mg/kg/day during post-partum stage leads to significantly reduction of somatic cell count in murrah buaffaloes than control group.

Kumar et al. (2012) studied the effect of Shatavari based polyherbal formulation at NDRI, Karnal and it was observed that significantly reduced the milk somatic cell counts in Karan fries cows.

4. Body condition score

Imtivati (2014) stated that average total BCS in Shatavari supplementation in Sahiwal cows during pre and post-partum stage leads to significant ($P<0.05$) improvement in BCS (3.99 ± 0.14) than control (3.85 ± 0.21).

Patel et al. (2013) observed the effect of two polyherbal galactogogue biscuits (19 g each) per day for initial 10 days of the month over a period of three months 22 lactating Surti buffaloes. They recorded that buffaloes of treatment group had lost significantly ($p<0.01$) lesser body condition scoring (BCS).



Materials

and

Methods

CHAPTER-3

MATERIALS AND METHODS

The aim of research study was to evaluate the effect of dietary supplementation of Shatavari root powder on milk yield, milk composition and haemato-biochemical parameters of lactating Sahiwal cows. In this chapter, a brief description of material used and experimental techniques adopted during the study has been given under following heads.

3.1 Location of the Experiment

The experiment was conducted within the premises of Livestock Farm Complex (LFC) of U.P. Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura. The elevation (altitude) of Mathura is 174 meters (570 feet) above the mean sea level and lies between 27⁰-14' to 27⁰-58' N longitude and 77⁰-71' to 78⁰-12' E latitude. The average annual rainfall varies between 532-620 mm. The climate is generally characterized as hot & dry during summers. The maximum temperature varies between 45 - 48⁰C and the min. temperature goes as down as up to 2⁰C during winters. The relative humidity varies from 18 to 79 %.

3.2 Selection and grouping of Sahiwal cows

The Sahiwal cows maintained at Livestock Farm Complex (LFC), College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura, were selected as experimental animals for present investigation. Eighteen healthy Sahiwal cows in their mid lactation were selected for the experiment. Experimental lactating cows were assigned into three groups (six lactating Sahiwal cow in each) on the basis of body weight and initial milk yield. The experiment protocol was in accordance of standard of Institute Animal Ethics committee and the approval of experimentation was also obtained from the committee. The average initial milk yield for group T1(control) was 7.45±0.11, for group T2 was 7.51±1.04 and for group T3 was 7.48±0.78 and the average body weight of group T1 was 341.83±13.01, for group T2 was 328.72±9.35 and for group T3 was 333.21±5.17. The selection and grouping of experimental cows are presented in Table 3.1.

Table 3.1: Selection and grouping of experimental Sahiwal cows

| Group | S. No | Animal No. | Body weight (kg) | Parity |
|----------------|--------------|-------------------|-------------------------|---------------|
| Control | 1 | S-268 | 415 | 2 |
| | 2 | S-299 | 349 | 4 |
| | 3 | S-168 | 320 | 3 |
| | 4 | S-174 | 370 | 5 |
| | 5 | SP-8 | 350 | 2 |
| | 6 | P-65 | 238 | 4 |
| | | Mean | 340.33 | |
| T1 | 1 | 295 | 329 | 3 |
| | 2 | 320 | 285 | 5 |
| | 3 | 272 | 357 | 3 |
| | 4 | 119 | 270 | 2 |
| | 5 | 142 | 350 | 4 |
| | 6 | 613 | 377 | 2 |
| | | Mean | 322.33 | |
| T2 | 1 | 294 | 340 | 4 |
| | 2 | 251 | 339 | 2 |
| | 3 | 624 | 360 | 3 |
| | 4 | 170 | 295 | 2 |
| | 5 | SP-46 | 315 | 3 |
| | 6 | P-50 | 345 | 5 |
| | | Mean | 332.33 | |

3.3 Housing and management of experimental Sahiwal cows

All the experimental cows were kept in loose housing system under standard management practice followed at LFC, DUVASU, Mathura. Experimental lactating cows were assigned into three groups (six lactating sahiwal cow in each) on the basis of their body weight and daily milk yield. They were kept along with their original herd mates in the same shed under loose housing system except at the time of milking. The feeding and other management practices for these cows remained same as was normally practiced for other cows of the herd. Feeding was done as per the ICAR (2013) standards. Diets were prepared by taking concentrates and roughage in proportion of 30: 70%. Roughage part is composed of mustard oil cake, gram chunni,

wheat bran, cotton seed cake, barley grain, mineral mixture. In green, sorghum and maize was offered.

3.4 Procurement, proximate analysis and supplementation of Shatavari root powder

The Shatavari root powder was procured from Kshipra Biotech private limited, Indore, Madhya Pradesh. Powder was kept in dry place to avoid contact with moisture and some amount of this powder was used for analysis of different proximate principles that is dry matter, crude protein, total ash, crude fibre and ether extract. Sahiwal cows were kept under the regular management practices at LFC, where two time milking is followed (morning and evening) and concentrate is generally fed to them at the time of milking. SRP was weighed using an electronic balance according to the doses (table 3.2) to be given in the respective group of the cows and the measured doses were kept in separate bags until feeding. Cows were fed with Shatavari root powder as per the doses properly mixed in the concentrate mixture provided before afternoon milking.

Table 3.2: Shatavari supplementation in experimental cows

| Groups | Diet |
|-------------------------|-------------------------------------|
| Control(T1) | Basal diet only, with no supplement |
| Treatment 1 (T2) | Basal diet and SRP@ 30gm/day/cow |
| Treatment 2 (T3) | Basal diets and SRP@ 60g/day/cow |

3.4 PARAMETERS STUDIED AND OBSERVATION TAKEN:

3.4.1 Proximate analysis

The representative samples of Shatavari root powder (SRP) analyzed for DM, CP, EE, CF and total ash (AOAC, 1990).

3.4.2 Dry matter (DM)

DM content in samples of feedstuffs and faeces was analyzed by using AOAC, (1990) method. 100 g sample was taken in a pre-weighed moisture cup or tray and it was placed in a hot air oven for 24:00 hours. The loss in moisture content after drying was estimated and DM was calculated as follows:

$$\text{DM (\%)} = \frac{\text{Weight of Sample after drying}}{\text{Weight of Sample taken}} \times 100$$

3.4.3 Crude protein (CP)

Apparatus: Kjeldahl flasks, digester, Kjeldahl distillation apparatus, Erlenmeyer flasks, titration assembly.

Reagents: Digestion mixture (Na_2SO_4 and CuSO_4 in the ratio of 9:1), 40% NaOH solution (400 g NaOH pellets dissolved in distilled water and volume made to 1000 ml), Concentrated H_2SO_4 (98% purity and specific gravity 1.84), 4% boric acid indicator solution (40 g boric acid dissolved to 1 L and added with 10 ml 0.2% bromocresol green and 20 ml 0.1% methyl red indicators) and N/10 H_2SO_4 solution.

Procedure

A known quantity of sample (about 0.5-1.0 g) was taken in digestion tube and digested with 20-30 ml concentrated H_2SO_4 and 2-3 g of digestion mixture till the solution became colourless. After digestion, the contents were cooled and volume was made to 250 ml. 10 ml of aliquot was distilled in Kjeldahl distillation apparatus (KELPLUS Nitrogen Analyzer, Chennai) after adding 10-15 ml of 40% NaOH solution. About 60-75 ml of distillate (light green colour) was collected into an Erlenmeyer flask containing 10 ml of 4% boric acid indicator solution. The distillate was then titrated against N/10 H_2SO_4 solution and the end point was recorded when colour changed to slight pinkish. Volume of N/10 H_2SO_4 solution used in titration was recorded.

Calculation

$$\text{N (\%)} = \frac{0.0014 \times 0.1 \times \text{Volume of N/10 } \text{H}_2\text{SO}_4 \text{ used} \times \text{Volume made (ml)}}{\text{Aliquot taken (ml)} \times \text{Sample taken (g)}} \times 100$$

The CP (%) of sample was calculated by multiplying the N content with factor 6.25 (protein contains 16% nitrogen).

3.4.4 Total ash (TA)

TA content in samples of feedstuffs and faeces was analyzed by using AOAC (1990) method. A known quantity of sample (about 10g) was taken in pre-weighed

silica crucible. After charring the sample on heater (till the smoke disappeared), the crucible was kept in muffle furnace for ignition at 550-600°C for 2-3 hours. Then the crucible was removed on cooling and kept in desiccator and weighed again to find out weight of ash. The ash content was calculated as given below

$$\text{TA (\%)} = \frac{\text{Weight of crucible + ash after drying} - \text{Weight of crucible}}{\text{Weight of sample taken}} \times 100$$

3.4.5 Crude fiber (CF)

Apparatus: Fibra plus (Pelican Equipments), suction apparatus, Gooch crucible, hot air oven, muffle furnace, desiccators, weighing balance.

Reagents: 1.25% H₂SO₄ and 1.25% NaOH

Procedure

Take the moisture and fat free sample of known quantity (after ether extraction). Weigh the samples accurately and note down the weights (W), transfer the weighed samples into oven dried crucible, place the crucible into the metal adapters of Fibra Plus hot extraction unit and ensure proper sealing of crucible against the adapter rubber.

Acid wash: Pre-heat the prepared solution of 1.25% H₂SO₄ in FIBRA STAT unit, pour 150 ml of 1.25 % H₂SO₄ into the extractors from the top for each sample. Switch on the instrument and set the temperature to 500°C, after boiling starts reduce the temperature at 400°C, allow the samples to boil for 30 minutes in acid. After 30 minutes of boiling, switch off the unit and drain the acid and wash the samples twice or thrice with distilled water. During draining, ensure that the knob is in vacuum mode, if the draining is not effective due to clogging of sample in the crucible, then, keep the knob in pressure mode.

Alkaliwash : Pre-heat the prepared solution of 1.25% NaOH in FIBRA STAT unit pour 150 ml of 1.25% NaOH into the extractors from the top for each sample. Switch on the instrument and set the temperature to 500°C, after boiling starts reduce the temperature to 400°C and allow the samples to boil for 30 minutes in alkali. After 30 minutes of boiling, switch off the unit and drain the alkali and wash the samples twice or thrice with distilled water. During draining, ensure that the knob is in vacuum mode, if the draining is not effective due to clogging of sample, then keep the knob in

pressure mode and press the pressure button twice or thrice and immediately turn the knob to vacuum mode. After alkali wash, take out crucibles and dry them in hot air oven at 100°C until the crucibles are free from moisture after that cool down the hot crucibles to room temperature by using a desiccator. Weigh the crucibles and record the readings (W_1) after that place all the crucibles in the muffle furnace at 400°C for ashing cool down the hot crucibles after ashing to room temperature using a desiccator, now weigh the crucibles and record the readings (W_2).

$$\text{CF (\%)} = \frac{\text{Weight of dried residue (} W_1 \text{)} - \text{Weight of ash (} W_2 \text{)}}{\text{Weight of sample taken}} \times 100$$

3.4.6 Ether extract (EE)

EE content in representative samples of feeds and fodders and faeces were analyzed by using AOAC (1990) method.

Apparatus: Socsplus extraction apparatus (Pelican Equipments), oil flask, thimble, hot air oven, desiccators, weighing balance.

Reagent: Petroleum ether (40-60°C).

Procedure

Rinse all the beakers and place them in oven with the temperature about 100°C and also the samples. If all moistures were removed from the beakers, place them in desiccators about 5 to 10 minutes to bring them into room temperature. Weigh the empty beaker and let the weight be W_1 . This is initial beaker weight (IBW). After that insert the thimble in the thimble holder and place it on the beaker and weigh the samples and transfer them to the thimble. Let the sample weight be (SW), sample weight may be 2 to 3 grams, pour the solvent in the beaker, the volume may be 80 to 100 ml, load the beakers in the system. Switch on the system and set the boiling temperature as the solvent's maximum boiling point, leave the process for about 60 minutes. After the process time, increase the temperature to recovery temperature range of 160 to 180°C, then rinse about 2 to 3 times in order to collect the remaining fat that may present in the sample or in the thimble, take out the beakers from the system then remove all the thimbles from the beaker and put the beakers into a hot air oven at 100°C, after 20 to 30 minutes, take out the beakers and place them in desiccators about 10 to 15 minutes for cooling up to the room temperature. Weigh the beakers, this is the finalweight of the beakers (FBW), let the weight be W_2 . By

substituting SW, W₁ and W₂ in the following formula, the percentage of fat present in the sample can be calculated.

$$EE (\%) = \frac{(\text{Weight of oil flask with ether extract } (W_2) - \text{Weight of oil flask } (W_1))}{\text{Weight of sample } (SW)} \times 100$$

3.5: MILK YIELD AND COMPOSITION:

3.5.1 Milk yield (L)

All lactating Sahiwal cows were milked twice and calves were used only for letdown of milk. Hand milking was performed twice daily (at 3:00 am and at 3:00 pm). Milk yield per animal was recorded on daily basis for 90 days. Milk yield was recorded with the help of electronic weighing balance of capacity 40 litres. After milking, milk was collected in milk can after screening through strainer.

3.5.2 Fortnightly milk yield (L) per animal per day

Milk yield per animal was also recorded at fortnightly interval (i.e., on 0, 15, 30, 45, 60, 75 and 90) days.

3.6 Milk composition

The representative samples of milk of individual cows were collected at fortnightly interval (i.e., 0, 15, 30, 45, 60, 75 and 90 day) for analysis of milk components i.e. milk fat, milk protein, milk lactose, SNF content and total milk solid. Each time 100 ml of milk sample was collected in sterile plastic bottle after cleaning and disinfecting teats and discharging the first streams of foremilk. Representative amount of each sample was used for estimation of milk components by Lactoscan MCCWS 3080 (ultrasonic milk analyzer).

3.7 Physio-chemical parameters of milk

- a. Temperature
- b. pH
- c. Density
- d. Specific gravity

For determination of physiochemical parameters 100 ml of representative sample was collected and analyzed with the help of Lactoscan MCCWS 3080 (ultrasonic milk analyzer).

3.8 Somatic cell count (SCC)

The somatic cell count of milk sample were analysed by Lactoscan somatic cell counter 6010.

Principle: The somatic cells in milk samples were stained with fluorescent dye (Sofia Green) which entered in cell nucleus and they turned intensive green. Advantage of fluorescent dye that it has weak background and has very high fluorescent intensity after it binds with DNA.

The somatic cell count (SCC) is a main indicator of milk quality. The majority of somatic cells are leukocytes which increase in numbers in milk as a response to a mastitis-causing pathogen.

- An individual cow SCC of 100,000 or less indicates an 'uninfected' cow.
- A threshold SCC of 200,000 would determine whether a cow is infected with mastitis.
- Cows with a result of greater than 200,000 are highly likely to be infected on at least one quarter.
- Cows infected with significant pathogens have an SCC of 300,000 or greater.

Essentially, a lower SCC indicates better animal health, as somatic cells originate only from inside the animal's udder. SCC monitoring is important because as the number of somatic cells increases, milk yield is likely to fall, primarily due to the damage to milk-producing tissue in the udder caused by mastitis pathogens and the toxins they produce, particularly when epithelial cells are lost.

3.9 Body condition score

The body condition score of all the eighteen lactating Sahiwal cows was evaluated at the beginning of experimentation and compared with BCS at the end of experimentation. BCS was done using 5 point scoring system. A score of 1 means cow is not in good body condition and is very thin, while 5 score designate an excessively fat cow, and an average body condition means a score of 3. Assessments of the experimental Sahiwal cows were done by three people at the beginning and at the end of the experiment.

Body condition score (BCS) basically describes the degree of fatness of an animal. The scoring system usually in use for dairy cattle is a 5-point scale, with 1

corresponding to an extremely thin cow, and 5 to a cow with excessive fat deposits (Wildman et. al., 1982).

For recording the body condition of the animals (a score given based on fat cover), following points were taken into account:

- a) Vertebral column (chine, loin and rump) flesh covering at the spinous processes of these regions.
- b) Spinous processes: their prominence and sharpness.
- c) Tail head region: prominence of depression between backbone and pins and between pins and hook bones.
- d) Ribs: their flesh covering.

Considering the above points, NRC (2001) presented a score card (Table 3.3), which was adopted in the present study. Body condition score of experimental cows were assessed by visual inspection and palpation once in very fortnight in trial.

Table 3.3: Body condition score card

| Criteria | Score 1 | Score 2 | Score 3 | Score 4 | Score 5 |
|-------------------------------|-----------------|-------------------|-----------------------|----------------|---------------------|
| Back bones | Prominent | Visible | Visible | Well covered | Very well Covered |
| Hips and shoulder bone | Prominent | Visible | Visible faintly | Not Visible | Showing fat Deposit |
| Ribs | Clearly Visible | Visible faintly | Generally not Visible | Well covered | Very well Covered |
| Tail -head area | Recessed | Slightly Recessed | Not recessed | Slightly lumpy | Very lumpy |
| Body outline | Skeletal | Bony | Almost smooth | Rounded | Bulging due to fat |

3.10 Haematological parameters

3.10.1. Collection of blood

Peripheral blood samples were collected in heparinized vacutainer tubes (BD Franklin, USA) by venipuncture of jugular vein at 0, 15, 30, 45, 60, 75 and 90 days post supplementation. Collected blood samples were analyzed for haematological, blood biochemical and hormonal attributes. A fraction of whole blood samples were used for haemoglobin (Hb) concentration, packed cell volume (PCV), Differential leucocyte count (DLC), Total leucocyte Count (TLC) and Total erythrocyte count (TEC). Remaining amount of blood samples was centrifuged at 3000 rpm for 15 min

to separate the plasma. Plasma samples were stored at -20°C until further analysis of metabolites.

3.10.2 Haemoglobin (Hb)

Haemoglobin concentration was estimated in the fresh blood with the help of Celltac alpha VET hematology analyser by Nihon Kohden MEK- 6550K.

3.10.3 Packed cell volume (PCV)

PCV was estimated in the fresh blood with the help of Celltac alpha VET hematology analyser by Nihon Kohden MEK- 6550K.

3.10.4 Differential leucocyte count (DLC)

DLC was estimated in the fresh blood with the help of Celltac alpha VET hematology analyser by Nihon Kohden MEK- 6550K.

3.10.5 Total leucocyte count (TLC)

TLC was estimated in the fresh blood with the help of Celltac alpha VET hematology analyser by Nihon Kohden MEK- 6550K.

3.10.6 Total erythrocyte count (TEC)

TEC was estimated in the fresh blood with the help of Celltac alpha VET hematology analyser by Nihon Kohden MEK- 6550K.

3.10.7 Beta hydroxyl butyric acid

Beta hydroxyl butyric acid was analysed with freestyle optium neo H meter. When the blood sample was applied to the test strip, the B-hydroxybutyrate in the blood reacted with the chemicals on the test strip, producing a small electrical current. This current was measured and then a result was displayed by the meter. The size of the current depends on the amount of beta hydroxyl butyric acid in the blood sample.

3.10.8 Blood glucose

The blood glucose was analysed by the Dr. Morepen Gluco One Blood Glucose Monitoring System. Glucose on the blood sample reacted with glucose oxidase (GOD) on the test strip and a harmless DC electrical current produced.

3.11 Biochemical parameters

Separated plasma samples, which were stored at -20°C were thawed and used for estimation of following blood biochemical attributes:

- a. Total protein
- b. Total albumen
- c. Cholesterol
- d. Triglycerides
- e. Urea/Creatinine
- f. Prolactin/Cortisol (Elisa Kit)

3.11.1 Plasma total protein

Total protein was estimated in blood plasma samples by using “Modified Biuret, End point assay test kit” supplied by ARKRAY Healthcare Pvt. Ltd. The principal of the assay is that the peptide bonds of proteins react with cupric ions in alkaline solution to form a coloured chelate; the absorbance was measured at 578 nm. The absorbance of final colour was proportional to the concentration of total protein in the sample.

Procedure

10 μl of plasma aliquot was pipette in 10 \times 75 mm tubes in duplicate, to which 1000 μl of working biuret reagent was added. Blank (10 μl distilled water) and standard (10 μl from protein standard 6.5 g/dl) was pipette in duplicates, to which 1000 μl of working biuret reagent was added. The contents were mixed well and incubated at room temperature for 1 minute. UV-spectrophotometer was blanked with reagent was added. The content was mixed well and incubated at room temperature for 1minute. UV- spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured at 578 nm.

Calculation: Total protein concentration was calculated as per formula and expressed in g/dL:

$$\text{Total protein (g/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 6.5$$

3.11.2. Plasma albumin

Albumin was estimated in blood plasma samples by using “Bromocresol green, End point assay test kit” supplied by ARKRAY Healthcare Pvt. Ltd. It is based

on the principal that albumin binds with anionic dye Bromocresol green (BCG) to form green colour complex, which is measured at 630 nm. Kit reagent were prepared and stored as per the instruction provided with the assay kit.

Procedure

10 µl of plasma aliquots were pipetted in 10×75 mm tubes in duplicate, to which 1000 µl of working albumin reagent was added. Blank (10 µl distilled water) and standard (10µl from standard 4g/dl) was pipetted in duplicates, to which 1000 µl of working albumin reagent was added. The content were mixed well and incubated at room temperature for 1 minute. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured at 630 nm.

Calculation: The albumin concentration was calculated as per the formula and expressed in g/dL:

$$\text{Albumin (g/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 4$$

3.11.3 Plasma cholesterol

Plasma cholesterol was estimated in plasma samples by “CHOD-PAP, End point assay test kit” supplied by ARKRAY Healthcare Pvt. Ltd. The principle of assay was cholesterol esters are hydrolyzed by cholesterol esterase to give free cholesterol and fatty acids. In subsequent reaction, cholesterol oxidase, oxidizes the 3-OH group of free cholesterol to liberate cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. Absorbance of coloured dye was measured at 505 nm and was proportional to amount of total cholesterol concentration in the sample.

Procedure

10 µl of plasma aliquots were pipetted in 10×75 mm tubes in duplicate, to which 1000 µl of working cholesterol reagent was added. Blank (10 µl distilled water) and standard (10 µl from standard 200 mg/dl) was pipetted in duplicate, to which 1000 µl of working cholesterol reagent was added. The content was mixed well and incubated at 37°C temperature for 10 minutes. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured at 505 nm.

Calculation: The cholesterol concentration was calculated as per the formula and expressed in mg/dL:

$$\text{Cholesterol (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

3.11.4 Plasma triglyceride

Plasma triglycerides were estimated in plasma samples by “End point assay test kit” supplied by ARKRAY Healthcare Pvt. Ltd. The principle of assay was triglycerides are hydrolyzed by lipoprotein lipase (LPL) to give glycerol and free fatty acids. In subsequent reaction, glycerol 3-PO₄ oxidase, oxidizes the 3-PO₄ group of free glycerol to liberate dihydroxy acetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide couples with 4-aminoantipyrine and 4-chlorophenol to produce red quinoneimine dye. Absorbance of coloured dye was measured at 505 nm and was proportional to amount of total triglycerides concentration in the sample.

Procedure

10 µl of plasma aliquots were pipetted in 10×75 mm tubes in duplicate, to which 1000 µl of working triglycerides reagent was added. Blank (10 µl distilled water) and standard (10 µl from standard 200 mg/dl) was pipetted in duplicate, to which 1000 µl of working triglycerides reagent was added. The contents were mixed well and incubated at 37°C temperature for 10 minutes. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured at 505 nm.

Calculation: The triglyceride concentration was calculated as per the formula and expressed in mg/dL:

$$\text{Triglyceride (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

3.11.5 Plasma urea

Plasma Urea were estimated in plasma samples by GLDH, “Initial rate assay test kit” supplied by Arkray Healthcare Pvt Ltd. The principle of assay was urea is hydrolysed in presence of Urease to produce Ammonia and carbon dioxide. The ammonia produced combines with a-Oxoglutarate and NADH in presence of GLDH (Glutamate Dehydrogenase) to yield Glutamate and NAD (Nicotinamide Adenine

dinucleotide). The decrease in absorbance due to consumption of NADH in unit time is proportional to the Urea nitrogen concentration in the sample and is measured at 340 nm.

Procedure

10 µl of plasma aliquots were pipetted in 10×75 mm tubes in duplicate, to which 1000 µl of working urea reagent was added. Blank (10 µl distilled water) and standard (10 µl from standard 50 mg/dl) was pipetted in duplicate, to which 1000 µl of working urea reagent was added. The contents were mixed well. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured at 340 nm.

Calculation: The urea concentration was calculated as per the formula and expressed in mg/dL:

$$\text{Urea (mg/dL)} = \frac{AT1 - AT2}{AS1 - AS2} \times 50$$

AT1: Initial O.D. of Test

AT2: Final O.D. of Test

AS1: Initial O.D. of standard

AS2: Final O.D. of Standard

3.11.6 Plasma creatinine

Creatinine reacts with Picric Acid in an alkaline medium to form an Orange coloured complex. The rate of formation of this complex is measured by reading the change in absorbance at 505 nm (490- 530 nm) in a selected interval of time and is proportional to the concentration of Creatinine. The reaction time and the concentration of Picric Acid and Sodium hydroxide have been optimised to avoid interference from keto-acids.

Procedure

100µl of plasma aliquots were pipetted in 10×75 mm tubes in duplicate, to which 1000 µl of working urea reagent was added. Blank (10 µl distilled water) and standard (10 µl from standard 2 mg/dl) was pipetted in duplicate, to which 1000 µl of working urea reagent was added. The contents were mixed well. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard

and test sample was measured at 505 nm. Initial absorbance of the Standard i.e. AS1 was measured after 30 seconds and final absorbance (AS2) after an interval of another 120 seconds. . After Standard reading, reading of Test i.e. AT1 and AT2 were taken accordingly (follow same procedure as for Standard).

Calculation: The creatinine concentration was calculated as per the formula and expressed in mg/dL:

$$\text{Creatinine (mg/dL)} = \frac{\text{AT2} - \text{AT1}}{\text{AS2} - \text{AS1}} \times 2$$

AT1: Initial O.D. of Test

AT2: Final O.D. of Test

AS1: Initial O.D. of standard

AS2: Final O.D. of Standard

3.11.7 Bovine prolactin

This kit is a enzyme-linked immune sorbent assay (ELISA). Add samples to the pre-coated plate. Then add biotinylated antigen. The antigens in the samples compete with the biotinylated antigen to bind to the capture antibody and incubate. Unbound antigen is washed away during a washing step. An avidin-HRP is then added and then it is incubated. Unbound avidin-HRP washed away during a washing step. TMB substrate is added and color develops. The reaction is stopped by addition of acidic stop solution and color changes into yellow that can be measured at 450 nm. The intensity of the color developed is inversely proportional to the concentration of PRL in the sample. The concentration of PRL in the sample is then determined by comparing the O.D. of the samples to the standard curve.

Reagents Preparation

- All reagents were brought to room temperature before use.
- Standard Reconstitute one vial of standard with 150 µl of Standard/Sample Diluent to generate a 16000 uIU/ml. Standard was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. Duplicate or triplicate standard points Prepare by serially diluting the standard stock solution 1: 2 with diluent to produce 8000 uIU/mL, 4000 uIU/mL, 2000 uIU/mL, 1000

ulU/ml and 500 ulU/mL. Standard/Sample diluents was added only as the zero standard (0 ulU/mL)

- Biotinylated Antigen: Briefly centrifuge the biotinylated antigen vial was briefly centrifuged and 1 ml biotinylated antigen was added to diluent and mix well. All this solution was pipetted back into the biotinylated antigen diluent vial to and 6 ml stock solution was generated. It was allowed to sit for 10 minutes with gentle agitation prior to making dilutions.
- Avidin-HRP Concentrate: Briefly low- speed centrifugation was done the avidin-HRP concentrates solution and then all avidin-HRP was pipette into the Avidin HRP Diluent vial. Proper mixing was done to generate a 6 ml stock solution. Allowed to sit for 10 minutes with gentle agitation prior to making dilutions.
- Wash Buffer Concentrate(25x): Dilute 20ml of concentrated wash buffer with 480 ml double distilled water to prepare 500 ml of wash buffer. If crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved.

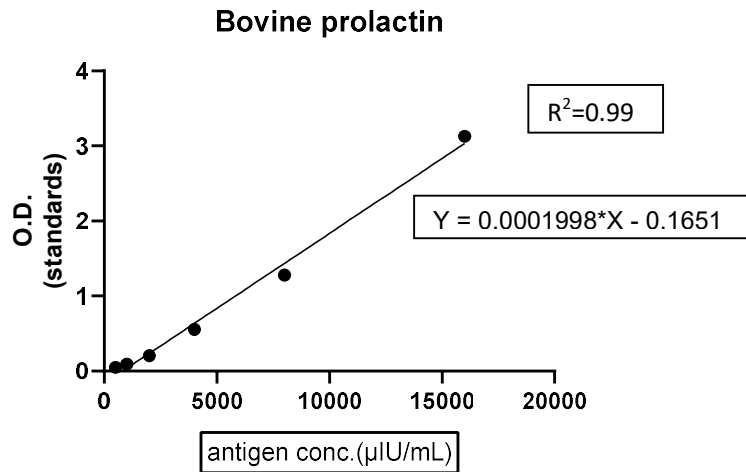
Assay procedure

- 1) Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
- 2) Blank wells: Only substrate solution A was added, substrate solution B and stop solution as blank control.
- 3) 50 µl diluted standard was added to standard well, 50 ul sample was added (Sample recommended dilution: 2-5 times when necessary) to the sample well, and 50 µl biotinylated antigen was added to each well. Mixed well. Plate was covered with a sealer and incubate for 60 minutes at 37°C.
- 4) The sealer was removed and, five times washing was performed with 300 µl wash buffer manually. Plate was inverted each time and the contents decanted; hitted 4-5 times on absorbent material to complete remove liquid.
- 5) 50 ul avidin-HRP was added to the standard well and sample well, the plate was covered with a sealer and incubated for 60 minutes at 37°C.
- 6) Sealer was removed and washed as described above.

- 7) 50 µl substrate solution A was added to each well and then 50 µl substrate solution B was also added to each well. Plate covered with a new sealer and incubated for 10 minutes at 37°C in the dark.
- 8) 50 µl Stop solution was added to each well, the blue color changed into yellow immediately.
- 9) Optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation

The duplicate readings were averaged for each standard, control, and sample. A standard curve was created by plotting the mean absorbance for each standard on the Y-axis against the target antigen concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration on the X axis versus the O.D of the standards on the Y axis and the best fit line can be determined by regression analysis. The linear equation ($X=Y+\text{Calibration Value}$) can be used to calculate the standard curve where X is the log of the concentration of the standard and Y is the OD value of the standard. If samples have been diluted (2-5 times is recommended) the concentration read from the standard curve must be multiplied by the dilution factor.



Standard curve of Prolactin

3.11.8 Bovine cortisol (ng/ml)

The DRG cortisol ELISA Kit is a solid phase enzyme-linked immune sorbent assay (ELISA), based on the principle of competitive binding.

End micro titre wells are coated with a monoclonal antibody directed towards an antigenic site on the cortisol molecule." Endogenous cortisol of a patient sample competes with a cortisol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of cortisol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of cortisol in the sample.

REAGENTS

Reagents provided

- 1) Micro titre wells, 12 x 8 (break apart) strips, 96 wells; wells coated with anti-cortisol antibody (monoclonal).
- 2) Standard (Standard Concentrations thus, corresponding to 0-6), 7 vials, 1 mL, ready to use; 0, 20, 50, 100, 200, 400, 800 ng/mL, 0, 55.2, 138, 276, 552, 1104, 2208 nmol/L.

Conversion factor: 1 ng/mL = 2.76 nmol/L. Contain non-mercury preservative.

- 3) Enzyme Conjugate, 1 vial, 25 mL, ready to use, cortisol conjugated to horseradish peroxidase; contains non-mercury preservative.
- 4) Substrate Solution, 1 vial, 14 mL, ready to use, tetra methyl benzidine (TMB).
- 5) Stop Solution, 1 vial, 14 mL, ready to use, contains 0.5 M H₂SO₄, avoid contact with the stop solution. It may cause skin irritations and burns. Wash Solution, 1 vial, 30 mL (40X concentrated)

Wash solution

Deionized water was added to the 40X concentrated wash solution. 30 mL of concentrated wash solution was diluted with 1170 ml deionized water to make a final volume of 1200 mL. The diluted wash solution is stable for 2 weeks at room temperature.

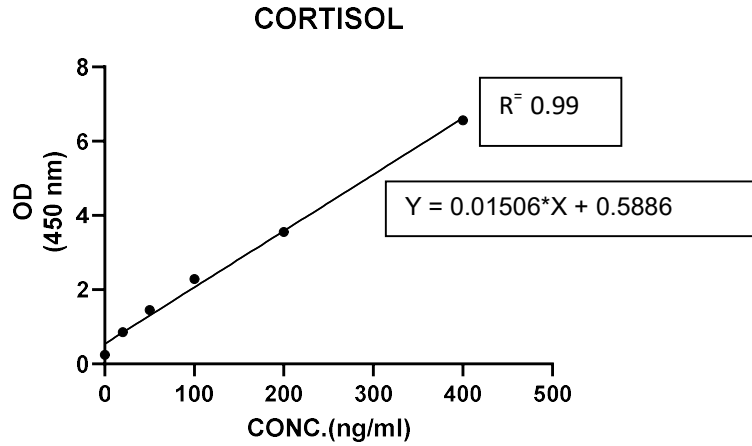
Procedure

Each run must include a standard curve.

- 1) Secure the desired number of micro titre wells in the frame holder.
- 2) 20 μL of each standard, control and sample was dispensed with new disposable tips into appropriate wells.
- 3) 200 μL enzyme conjugate was dispensed into each well and thoroughly mixed for 10 seconds. Complete mixing was performed in this step and then Incubation was done for 60 minutes at room temperature.
- 4) Briskly shaking done to out the contents of the wells. The wells were rinsed 3 times with diluted wash solution (400 μL per well) the wells were struck sharply on absorbent paper to remove residual droplets.
- 5) 100 μL of Substrate Solution was added to each well
- 6) 15 minutes incubation was done at room temperature.
- 7) Enzymatic reaction was stopped by adding 100 μL of stop solution to each well.
- 8) The absorbance (OD) of each well was determined at 450 ± 10 nm with a micro titre plate reader.
- 9) Wellswere read within 10 minutes after adding the stop solution.

Calculation

- 1) Average absorbance was calculated for the values for each set of standards, control and samples.
- 2) Using semi-logarithmic graph paper construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (YT-axis and concentration on the horizontal (X) axis.
- 3) Using the mean absorbance value for each sample corresponding concentration was determined from the standard curve.
- 4) Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter Rodbard or 4 Parameter Marquardt)
- 5) The concentration of the samples can be read directly from this standard curve Samples with concentrations higher than that of the highest standard have to be further diluted or reported as 800 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.



Standard curve of Cortisol

3.11.9 Statistical analysis

The data recorded for different parameters were analyzed as per the standard statistical procedure (Snedecor and Cochran, 1980) using SPSS 20 version. The Mean \pm SE values for the different parameters were compared among treatment groups using Duncan multiple range test (Duncan, 1955). Significant differences were considered at p level (<0.05).



Fig. 3.1: Shatavari roots



Fig. 3.2: Shatavari root powder



Fig. 3.3: Automatic Hematology Analyzer

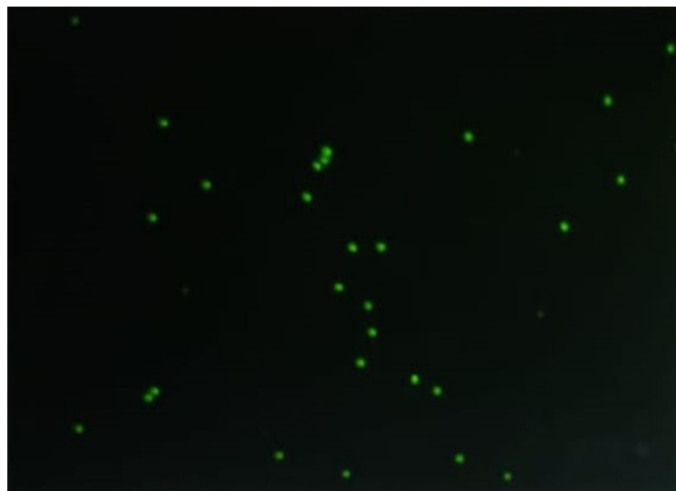


Fig. 3.4: Somatic cells in milk sample



Fig. 3.5: Lactoscan milk composition analyzer

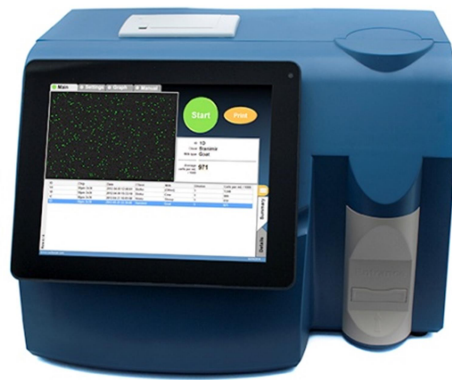


Fig. 3.6: Somatic cell counter



Fig. 3.7: Automatic biochemical analyzer



Fig.3.8: Performing biochemical test

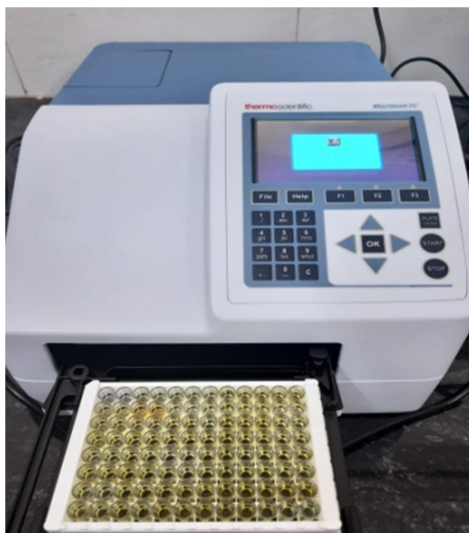


Fig.3.9:ELISA Plate with ELISA reader



Fig. 3.10: collecting blood sample

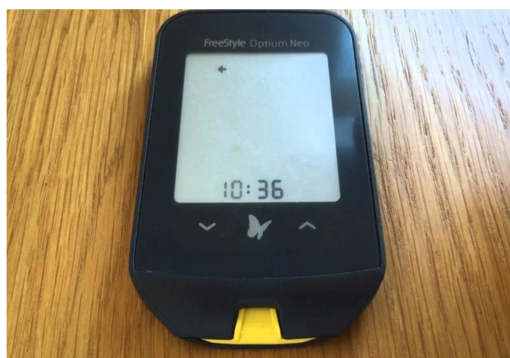


Fig. 3.11: Blood ketone body reader



Results



The objectives of the study were to evaluate the effect of dietary supplementation of Shatavari root powder on milk yield, milk composition and haemato-biochemical parameters of lactating Sahiwal cows. In this experiment, T1 group was taken as control which was not supplemented with Shatavari root powder, T2 group was supplemented with Shatavari root powder @ 30g/cow/day and T3 group was supplemented with Shatavari root powder @ 60g/cow/day. The observations recorded on different parameters have been analyzed and are presented in this chapter.

4.1 Proximate composition (% DM basis) of Shatavari root powder

The chemical composition of Shatavari root powder (on dry matter basis) has been presented in Table 4.1. The proximate principle i.e., dry matter, crude protein, total ash, crude fibre and ether extract in SRP were found to be 91.3, 4.12, 9.5, 10.58 and 0.82 % respectively.

Table 4.1: Proximate composition (%DM basis) of Shatavari root powder fed to lactating Sahiwal cows

| S. No. | Parameters | <i>A. racemosus</i> root powder |
|---------------|-------------------|--|
| 1. | DM | 91.3% |
| 2. | CP | 4.12% |
| 3. | Total Ash | 9.5% |
| 4. | CF | 10.58% |
| 5. | EE | 0.82% |

4.2 Daily milk yield (Litre) per animal per day and at fortnight intervals

The daily milk yield (litre) of experimental animals at weekly interval have been presented in Table 4.2 & Fig. 4.1.

Overall milk yield of treatment groups was found significantly ($P<0.05$) higher than the Control group.

The overall mean value of milk yield during the experimental period in T1, T2 and T3 groups 6.02 ± 0.19 , 6.89 ± 0.23 and 7.48 ± 0.78 respectively, there was significantly ($P<0.05$) higher milk yield in the T2 and T3 than the control (T1).

T2 (6.89 ± 0.23) and T3 (7.37 ± 0.12) were not found to be differed significantly ($P<0.05$) in milk yield however, numerically milk yield (litre) was higher in T3 compared to T2.

Table 4.2: Average daily milk yield (Litre) during different weeks

| Week | T1 (Control) | T2 | T3 |
|--------------------------|-----------------|--------------------|-----------------|
| 0 | 7.45 ± 0.11 | 7.51 ± 1.04 | 7.48 ± 0.78 |
| 1 | 7.07 ± 0.17 | 7.38 ± 1.03 | 7.24 ± 0.49 |
| 2 | 7.28 ± 0.20 | 7.75 ± 0.95 | 7.87 ± 0.51 |
| 3 | 6.35 ± 0.72 | 7.07 ± 0.94 | 7.77 ± 1.03 |
| 4 | 6.47 ± 0.77 | 7.32 ± 0.96 | 7.39 ± 0.33 |
| 5 | 6.28 ± 0.76 | 7.09 ± 0.90 | 7.25 ± 0.21 |
| 6 | 5.89 ± 0.77 | 6.75 ± 0.82 | 7.54 ± 0.43 |
| 7 | 5.71 ± 0.71 | 6.73 ± 0.82 | 7.54 ± 0.47 |
| 8 | 5.54 ± 0.66 | 6.63 ± 0.78 | 7.35 ± 0.49 |
| 9 | 5.50 ± 0.68 | 6.57 ± 0.81 | 7.38 ± 0.48 |
| 10 | 5.26 ± 0.69 | 6.41 ± 0.78 | 7.21 ± 0.36 |
| 11 | 4.89 ± 0.71 | 6.28 ± 0.77 | 6.97 ± 0.43 |
| 12 | $4.60^a\pm0.70$ | $6.05^{ab}\pm0.75$ | $6.89^b\pm0.59$ |
| Overall (Mean±SE) | $6.02^a\pm0.19$ | $6.89^b\pm0.23$ | $7.37^b\pm0.12$ |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at $P<0.05$

4.2.11 Fortnightly milk yield (Litre) per animal per day

The milk yield (Litre) of experimental animals at fortnightly interval have been presented in **Table 4.3 & Fig. 4.2**. Overall milk yield of treatment groups was found significantly ($P<0.05$) higher than the Control group.

The overall mean value of milk yield during the experimental period in T1, T2 and T3 groups 6.06 ± 0.26 , 6.83 ± 0.32 and 7.35 ± 0.17 respectively, there was significantly ($P<0.05$) higher milk yield in the T2 and T3 than the control (T1).

Table 4.3: Effect of Shatavari root powder on average milk yield (litre) over fortnights

| Days | T1 (control) | T2 | T3 |
|--------------------------|-------------------------|--------------------------|-------------------------|
| 0 | 7.46±0.11 | 7.52±1.04 | 7.48±0.78 |
| 15 | 7.20±0.16 | 7.15±0.95 | 7.51±0.36 |
| 30 | 6.39±0.80 | 7.15±0.95 | 7.51±0.36 |
| 45 | 5.99±0.77 | 6.89±0.85 | 7.44±0.32 |
| 60 | 5.57±0.67 | 6.65±0.81 | 7.40±0.49 |
| 75 | 5.25±0.70 | 6.44±0.79 | 7.20±0.37 |
| 90 | 4.56 ^a ±0.71 | 6.01 ^{ab} ±0.76 | 6.91 ^b ±0.58 |
| Overall (Mean±SE) | 6.06 ^a ±0.26 | 6.83 ^b ±0.32 | 7.35 ^b ±0.17 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at $P<0.05$

4.3. Milk Composition Parameters of Milk

4.3.1. Milk fat (%)

The milk fat % of experimental animals at fortnightly interval have been presented in **Table 4.4**. The values ranged from 3.25% to 4.04% in T1 (Control), 3.38% to 4.09% in T2, 3.34% to 4.06% in T3 group. No significant difference ($P>0.05$) was observed in fat percent among different treatment groups over different fortnights. The overall mean value of milk fat percent during the experimental period in T1, T2 and T3 groups were 3.50 ± 0.05 , 3.61 ± 0.07 and 3.67 ± 0.06 % respectively.

Table 4.4: Effect of Shatavari root powder on average milk fat (%) over fortnights

| Days | T1 (Control) | T2 | T3 |
|----------------------|--------------|-----------|-----------|
| 0 | 3.25±0.06 | 3.38±0.05 | 3.34±0.33 |
| 15 | 3.38±0.05 | 3.44±0.12 | 3.40±0.06 |
| 30 | 3.30±0.04 | 3.45±0.11 | 3.47±0.02 |
| 45 | 3.48±0.09 | 3.41±0.09 | 3.45±0.06 |
| 60 | 3.50±0.11 | 3.67±0.28 | 3.76±0.15 |
| 75 | 3.52±0.08 | 3.84±0.18 | 3.98±0.17 |
| 90 | 4.04±0.21 | 4.09±0.23 | 4.06±0.17 |
| Overall (Mean±SE) | 3.50±0.05 | 3.61±0.07 | 3.67±0.06 |

4.3.2. Milk protein (%)

The milk Protein % of experimental animals at fortnightly interval have been presented in **Table 4.5**. The values ranged from 3.15 to 3.57 % in T1 (Control), 3.05 % to 3.36 in T2 and 3.23% to 3.38 % in T3 groups. No significant difference ($P>0.05$) was observed in protein percent among different treatment groups over different fortnights.

The overall mean value of milk protein percent during the experimental period in T1, T2 and T3 groups were 3.30±.05, 3.25±.04 and 3.28±.02 percent respectively,

Table 4.5: Effect of Shatavari root powder on average milk protein (%) over fortnights

| Days | T1 (Control) | T2 | T3 |
|----------------------|--------------|-----------|-----------|
| 0 | 3.15±0.08 | 3.05±0.24 | 3.27±0.06 |
| 15 | 3.38±0.05 | 3.36±0.05 | 3.38±0.03 |
| 30 | 3.38±0.03 | 3.36±0.04 | 3.38±0.02 |
| 45 | 3.34±0.04 | 3.34±0.04 | 3.29±0.09 |
| 60 | 3.22±0.04 | 3.17±0.09 | 3.23±0.05 |
| 75 | 3.17±0.07 | 3.28±0.04 | 3.27±0.04 |
| 90 | 3.57±0.36 | 3.22±0.05 | 3.24±0.03 |
| Overall (Mean±SE) | 3.30±0.05 | 3.25±0.04 | 3.28±0.02 |

4.3.3. Milk solid not fat value (%)

The milk SNF % of experimental animals at fortnightly interval have been presented in **Table 4.6 & Fig. 4.3**. The values ranged from 7.55 to 8.38% in T1 (Control), 7.65 to 8.66 % T2 and 7.64 to 9.06 % in T3 groups.

The milk SNF % of treatment groups T2 and T3 was found significantly higher ($P<0.05$) than the Control group (T1) during 30,45,60,75 and 90 day.

The overall Mean \pm SE value of milk SNF percent during the experimental period in T1, T2 and T3 groups were 7.81 ± 0.05 , 8.07 ± 0.05 and 8.20 ± 0.07 % respectively.

Table 4.6: Effect of Shatavari root powder on average milk solid not Fat (%) over fortnights

| DAYS | T1 (Control) | T2 | T3 |
|---|------------------------------|------------------------------|------------------------------|
| 0 | 7.55 \pm 0.01 | 7.65 \pm 0.05 | 7.64 \pm 0.04 |
| 15 | 7.56 \pm 0.01 | 7.66 \pm 0.05 | 7.69 \pm 0.05 |
| 30 | 7.64 ^a \pm 0.06 | 7.90 ^b \pm 0.04 | 8.01 ^b \pm 0.02 |
| 45 | 7.73 ^a \pm 0.09 | 8.09 ^b \pm 0.02 | 8.20 ^b \pm 0.03 |
| 60 | 7.89 ^a \pm 0.08 | 8.21 ^b \pm 0.02 | 8.3 ^b \pm 0.03 |
| 75 | 7.92 ^a \pm 0.08 | 8.33 ^b \pm 0.02 | 8.54 ^c \pm 0.05 |
| 90 | 8.38 ^a \pm 0.13 | 8.66 ^a \pm 0.07 | 9.06 ^b \pm 0.15 |
| Overall (Mean\pmSE) | 7.81 ^a \pm 0.05 | 8.07 ^b \pm 0.05 | 8.20 ^b \pm 0.07 |

Values (Mean \pm SE) within same row for a particular parameter bearing different superscript differed significantly at $P<0.05$

4.3.4. Milk lactose (%)

The milk lactose % of experimental animals at fortnightly interval have been presented in **Table 4.7& Fig. 4.4**. The values ranged from 4.06 to 4.68 % in T1 (Control), 4.36 to 5.01% in T2, 4.39 to 5.24% in T3 groups.

The overall mean value of milk lactose percent during the experimental period in T1, T2 and T3 groups were 4.47 ± 0.05 , 4.80 ± 0.06 and 5.0 ± 0.06 % respectively, there was significant ($P<0.05$) difference between T1(control), T2 and T3 groups.

The statistical analysis of data revealed significant ($P<0.05$) effect on milk lactose due to Shatavari supplementation. Maximum milk lactose was observed in T3 group followed by T2 group.

Table 4.7: Effect of Shatavari root powder on average milk lactose (%) over fortnights

| Days | T1(Control) | T2 | T3 |
|--------------------------|------------------------------|------------------------------|------------------------------|
| 0 | 4.26±0.08 | 4.36±0.30 | 4.39±0.26 |
| 15 | 4.06 ^a ±0.22 | 4.55 ^{ab} ±0.18 | 4.73 ^b ±0.13 |
| 30 | 4.36 ^a ±0.09 | 4.79 ^b ±0.11 | 5.07 ^c ±0.02 |
| 45 | 4.65 ^a ±0.10 | 4.94 ^b ±0.03 | 5.21 ^c ±0.01 |
| 60 | 4.68 ^a ±0.10 | 4.98 ^b ±0.04 | 5.24 ^c ±0.01 |
| 75 | 4.65 ^a ±0.11 | 4.99 ^b ±0.04 | 5.20 ^b ±0.02 |
| 90 | 4.66 ^a ±.11 | 5.01 ^b ±0.04 | 5.18 ^b ±0.05 |
| Overall (Mean±SE) | 4.47^a±0.05 | 4.80^b±0.06 | 5.0^c ±0.06 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at $P<0.05$

4.3.5. Total milk solids (%)

The total milk solids % of experimental animals at fortnightly interval have been presented in **Table 4.8** & **Fig.4.5**. The value ranged from 9.92 to 10.65 % for T1 (control), 10.12 to 12.90 % in T2 and 10.50 to 13.82 % in T3.

Overall mean value of total milk solids % during the experimental period in T1, T2 and T3 groups were 10.31±0.06, 11.53±0.17 and 12.98±0.19 % respectively, there was significant ($P<0.05$) difference observed between T1(control), T2 and T3 groups.

Table 4.8 Effect of Shatavari root powder on average total milk solids (%) over fortnights

| Days | T1 (control) | T2 | T3 |
|--------------------------|-------------------------------|-------------------------------|-------------------------------|
| 0 | 9.92 ^a ±0.13 | 10.12 ^{ab} ±0.22 | 10.50 ^b ±0.14 |
| 15 | 10.22 ^a ±0.19 | 10.93 ^a ±0.34 | 12.20 ^b ±0.12 |
| 30 | 10.26 ^a ±0.10 | 11.08 ^b ±0.35 | 12.97 ^c ±0.22 |
| 45 | 10.22 ^a ±0.11 | 11.70 ^b ±0.55 | 13.50 ^c ±0.31 |
| 60 | 10.65 ^a ±0.15 | 12.14 ^b ±0.13 | 13.82 ^c ±0.11 |
| 75 | 10.33 ^a ±0.14 | 11.88 ^b ±0.31 | 14.04 ^c ±0.03 |
| 90 | 10.56 ^a ±0.13 | 12.90 ^b ±0.22 | 13.82 ^c ±0.17 |
| Overall (Mean±SE) | 10.31^a±0.06 | 11.53^b±0.17 | 12.98^c±0.19 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at P<0.05

4.4. Physio-chemical Parameters of Milk

4.4.1. Milk temperature (°C)

The variation of temperature (°C) over days at every fortnightly interval have been presented in **Table 4.9**. The temperature varied from 34.27°C to 36.86°C in T1 (Control), 34.31°C to 37.08°C in T2 and 34.26°C to 36.86°C in T3 groups. No effect of Shatavari was observed on milk temperature.

The overall mean value of milk temperature during the experimental period in T1, T2 and T3 groups were 35.19±0.15°C, 35.21±0.16°C and 35.18±0.15°C, respectively. The statistical analysis of data revealed no significant difference (p>0.05) between T1 (control), T2 and T3 groups.

Table 4.9: Effect of Shatavari root powder on average milk temperature (°C) over fortnights:

| Days | T1 (Control) | T2 | T3 |
|--------------------------|-------------------|-------------------|-------------------|
| 0 | 36.86±0.12 | 37.08±0.11 | 36.86±0.17 |
| 15 | 34.46±0.12 | 34.68±0.16 | 34.56±0.21 |
| 30 | 36.22±0.09 | 36.01±0.13 | 36.05±0.18 |
| 45 | 35.28±0.05 | 35.23±0.23 | 35.25±0.12 |
| 60 | 34.27±0.26 | 34.31±0.16 | 34.26±0.08 |
| 75 | 34.41±0.21 | 34.51±0.25 | 34.64±0.20 |
| 90 | 34.84±0.15 | 34.65±0.25 | 34.64±0.21 |
| Overall (Mean±SE) | 35.19±0.15 | 35.21±0.16 | 35.18±0.15 |

4.2. Milk pH:

The variation of pH over days at every fortnightly interval have been presented in **Table 4.10**. The pH varied from 7.20 to 7.27 in T1 (Control), 7.13 to 7.26 in T2 and 7.11 to 7.31 in T3 groups.

The overall mean value of pH during the experimental period in T1, T2 and T3 groups were 7.23 ± 0.01 , 7.21 ± 0.02 and 7.23 ± 0.02 , respectively, there was no significance difference ($p > 0.05$) observed between T1 (control), T2 and T3 groups.

Thus, there was non-significant effect of Shatavari supplementation on pH of milk.

Table 4.10: Effect of Shatavari root powder on average milk pH over fortnights

| Days | T1 (control) | T2 | T3 |
|--------------------------|---------------------|-----------|-----------|
| 0 | 7.22±0.01 | 7.19±0.03 | 7.18±0.04 |
| 15 | 7.22±0.02 | 7.26±0.02 | 7.24±0.05 |
| 30 | 7.20±0.02 | 7.23±0.05 | 7.31±0.14 |
| 45 | 7.23±0.02 | 7.13±0.02 | 7.21±0.03 |
| 60 | 7.27±0.03 | 7.23±0.01 | 7.26±0.01 |
| 75 | 7.24±0.02 | 7.20±0.04 | 7.28±0.03 |
| 90 | 7.21±0.02 | 7.22±0.07 | 7.11±0.05 |
| Overall (Mean±SE) | 7.23±0.01 | 7.21±0.02 | 7.23±0.02 |

4.4.3. Density (g/ml)/Relative density of milk over fortnights:

The variation of milk density (**g/ml**) over days at every fortnightly interval have been presented in **Table 4.11**. The density varied from 29.78 to 33.02 in T1 (Control), 29.37 to 33.48 in T2 and 30.05 to 33.38 in T3 groups.

The overall mean value of density during the experimental period in T1, T2 and T3 groups were 31.46 ± 0.50 , 31.55 ± 0.55 and 31.68 ± 0.34 , respectively, there was non-significant ($p > 0.05$) difference observed between T1 (control), T2 and T3 groups.

Considering the density of water as 1gm/ml the specific gravity of milk is remain same as the density of milk.

Table 4.11: Effect of Shatavari root powder on average density of milk over fortnights.

| Days | T1 (Control) | T2 | T3 |
|------------------------------|-------------------|-------------------|-------------------|
| 0 | 29.78±1.36 | 29.37±3.43 | 31.96±0.70 |
| 15 | 31.55±1.11 | 32.40±0.77 | 32.50±0.43 |
| 30 | 33.02±0.56 | 33.48±0.55 | 33.38±0.33 |
| 45 | 32.58±0.42 | 32.71±0.62 | 31.54±1.76 |
| 60 | 30.53±0.93 | 30.83±0.36 | 30.84±0.75 |
| 75 | 30.41±0.90 | 32.36±0.56 | 31.51±0.78 |
| 90 | 32.39±2.73 | 29.72±0.76 | 30.05±0.41 |
| Overall (Mean±SE) | 31.46±0.50 | 31.55±0.55 | 31.68±0.34 |

4.5. Somatic cell count (*10³cells/ml) of milk:

The somatic cell count of milk (*10³cells/ml) of experimental animals at fortnightly interval have been presented in **Table 4.12**.

SCC ranged from 125 to 132 (*10³cells/ml milk), 130 to 107 (*10³cells/ml milk) and 121 to 64 (*10³cells/ml milk) respectively in T1 (control), T2 and T3 at first and last fortnight.

Overall milk SCC in the T3 group was 97 (×10³cells/ml) which was significantly (P<0.05) lower than the T2 (107×10³cells/ml) and T1 (130×10³cells/ml).

Table 4.12: Effect of Shatavari root powder on average Somatic cell count (×10³cells/ml) of milk over fortnights:

| Days | T1 (Control) | T2 | T3 |
|------------------------------|---------------------------|---------------------------|--------------------------|
| 0 | 125±09 | 130±05 | 121±08 |
| 15 | 127±08 | 125±07 | 117±07 |
| 30 | 127±07 | 119±07 | 110±05 |
| 45 | 132 ^b ±07 | 107 ^{ab} ±10 | 102 ^a ±06 |
| 60 | 133 ^b ±07 | 99 ^a ±11 | 91 ^a ±08 |
| 75 | 137 ^b ±06 | 89 ^a ±13 | 74 ^a ±10 |
| 90 | 132 ^b ±06 | 76 ^a ±17 | 64 ^a ±10 |
| Overall (Mean±SE) | 130^b±02 | 107^b±04 | 97^a±04 |

4.6. Body Condition Score:

The BCS of experimental animals at 0 day and 90th and have been described in **Table 4.13 & Fig. 4.6**. The BCS ranged from 2.79 to 2.85 in T1 (Control), 2.84 to 2.96 in T2 and 2.83 to 3.05 in T3 groups.

The overall mean value of body condition score during the experimental period in T1, T2 and T3 groups were respectively 2.82, 2.90 and 2.94. No significant ($p>0.05$) difference was observed between T1 (control), T2 and T3 groups; but numerically T3 has the highest value among the treatment groups.

Table 4.13: Effect of Shatavari root powder on average body condition score:

| Days | T1 (Control) | T2 | T3 |
|--------------------------|--------------|-----------|-----------|
| 0 | 2.85±0.06 | 2.84±0.30 | 2.83±0.08 |
| 90 | 2.79±0.10 | 2.96±0.12 | 3.05±0.04 |
| Overall (Mean±SE) | 2.82±0.04 | 2.90±0.08 | 2.94±0.05 |

4.7. Haematological parameters

4.7.1. Haemoglobin (g/dL)

Hemoglobin value (g/dL) of different groups have been presented in **Table 4.14**. The Hemoglobin (g/dL) ranged 9.33 to 9.60 in T1 (Control), 9.53 to 9.67 in T2 and 9.70 to 10.08 in T3 group.

The Overall Mean±SE values were T1 (9.48±0.15), T2 (9.59±0.12) and T3 (9.83±0.09). No significant difference ($p>0.05$) was observed in haemoglobin among different treatment groups over different fortnights.

Table 4.14: Effect of Shatavari root powder on average haemoglobin (g/dl) over fortnights

| Days | T1 (control) | T2 | T3 |
|--------------------------|--------------|-----------|------------|
| 0 | 9.48±0.47 | 9.53±0.41 | 9.63±0.37 |
| 15 | 9.60±0.49 | 9.67±0.31 | 9.73±0.18 |
| 30 | 9.43±0.42 | 9.63±0.30 | 9.72±0.20 |
| 45 | 9.33±0.38 | 9.53±0.33 | 9.70±0.15 |
| 60 | 9.40±0.37 | 9.55±0.43 | 9.93±0.26 |
| 75 | 9.53±0.48 | 9.58±0.35 | 10.03±0.25 |
| 90 | 9.55±0.39 | 9.62±0.34 | 10.08±0.28 |
| Overall (Mean±SE) | 9.48±0.15 | 9.59±0.12 | 9.83±0.09 |

4.7.2 PCV (%)

PCV % of different groups have been presented in **Table 4.15**. The overall mean values were T1 (28.4±0.46), T2 (28.6±0.37) and T3 (29.5±0.27). No significant ($p>0.05$) difference was observed in PCV percent among different treatment groups over different fortnights.

Table 4.15: Effect of Shatavari root powder on average PCV (%) over fortnights:

| Days | T1 (Control) | T2 | T3 |
|--------------------------|--------------|-----------|-----------|
| 0 | 28.5±1.42 | 28.6±1.22 | 28.9±1.11 |
| 15 | 28.8±1.48 | 29±0.92 | 29.2±0.55 |
| 30 | 28.3±1.27 | 28.9±0.90 | 29.2±0.59 |
| 45 | 28±1.15 | 28.6±1.00 | 29.1±0.44 |
| 60 | 28.2±1.11 | 28.7±1.30 | 29.8±0.78 |
| 75 | 1.43±1.43 | 28.8±1.06 | 30.1±0.75 |
| 90 | 28.7±1.17 | 28.9±1.03 | 30.3±0.83 |
| Overall (Mean±SE) | 28.4±0.46 | 28.6±0.37 | 29.5±0.27 |

4.7.3. Differential Leucocyte Count

4.7.3.1. Lymphocyte Count(%)

Lymphocyte % of different groups have been presented in **Table 4.16**. The overall mean values were T1 (56.16±1.23), T2 (57.94±0.90) and T3 (59.11±0.98). No significant ($p>0.05$) difference was observed in the T1, T2 and T3 but overall numerical value was higher in the T3.

However, lymphocyte (%) were found to be in normal physiological range.

Table 4.16: Effect of Shatavari root powder on average lymphocyte count (%) over fortnights.

| Days | T1(control) | T2 | T3 |
|--------------------------|-------------|------------|------------|
| 0 | 59.07±2.96 | 59.57±2.29 | 59.73±2.34 |
| 15 | 57.90±3.06 | 57.05±2.14 | 59.23±1.93 |
| 30 | 53.72±2.72 | 56.13±1.15 | 57.60±2.40 |
| 45 | 55.98±4.21 | 58.12±3.57 | 61.17±3.22 |
| 60 | 55.05±3.33 | 60.22±3.18 | 58.52±3.20 |
| 75 | 56.85±3.64 | 58.07±1.91 | 63.35±1.99 |
| 90 | 54.58±3.79 | 56.45±2.35 | 54.17±2.40 |
| Overall (Mean±SE) | 56.16±1.23 | 57.94±0.90 | 59.11±0.98 |

4.7.3.2. Eosinophil Count (%)

Eosinophil % of different groups have been presented in **Table 4.17**. The overall mean values were T1 (3.60±0.37), T2 (3.47±0.26) and T3 (3.72±0.45). No significant difference ($p>0.05$) was observed in the T1, T2 and T3 but they were in normal physiological range in all the groups.

Table 4.17: Effect of Shatavari root powder on average eosinophil count (%) over fortnights

| Days | T1 (control) | T2 | T3 |
|------------------------------|--------------|-----------|-----------|
| 0 | 3.37±1.07 | 4.43±0.68 | 2.22±0.31 |
| 15 | 2.83±0.47 | 3.18±1.34 | 2.22±0.62 |
| 30 | 4.08±0.63 | 3.05±0.47 | 3.48±0.44 |
| 45 | 2.97±0.76 | 3.52±0.30 | 8.65±1.86 |
| 60 | 4.10±1.35 | 3.03±0.33 | 2.52±0.52 |
| 75 | 2.82±0.34 | 2.72±0.28 | 2.38±0.49 |
| 90 | 5.20±1.65 | 4.37±0.81 | 4.55±0.75 |
| Overall (Mean±SE) | 3.60±0.37 | 3.47±0.26 | 3.72±0.45 |

4.7.3.3. Monocyte Count (%)

Monocyte % of different groups have been presented in **Table 4.18**. The overall mean values for T1 (0.35±0.03), T2 (0.35±0.05) and T3 (0.40±0.06). No significant difference ($p>0.05$) was observed in the T1, T2 and T3.

Table 4.18: Effect of Shatavari root powder on average monocyte count (%) over fortnights:

| Days | T1(control) | T2 | T3 |
|------------------------------|-------------|-----------|-----------|
| 0 | 0.43±0.03 | 0.48±0.11 | 0.27±0.08 |
| 15 | 0.32±0.07 | 0.27±0.08 | 0.60±0.17 |
| 30 | 0.35±0.06 | 0.30±0.09 | 0.58±0.25 |
| 45 | 0.43±0.10 | 0.48±0.22 | 0.33±0.17 |
| 60 | 0.20±0.07 | 0.20±0.11 | 0.22±0.03 |
| 75 | 0.48±0.12 | 0.37±0.09 | 0.47±0.19 |
| 90 | 0.20±0.07 | 0.35±0.14 | 0.33±0.13 |
| Overall (Mean±SE) | 0.35±0.03 | 0.35±0.05 | 0.40±0.06 |

4.7.3.4. Granulocyte Count (%)

Granulocyte % of different groups have been presented in **Table 4.19**. The overall mean values were T1 (40.05±1.12), T2 (38.57±1.04) and T3 (37.82±1.07). No significant ($p>0.05$) difference was observed in the T1, T2 and T3.

Table 4.19: Average granulocyte count (%) over fortnights:

| Days | T1 (Control) | T2 | T3 |
|--------------------------|--------------|------------|------------|
| 0 | 38.22±1.75 | 35.52±2.19 | 39.00±1.74 |
| 15 | 39.03±2.86 | 38.92±3.56 | 37.90±1.32 |
| 30 | 41.85±2.54 | 40.52±1.28 | 38.33±2.44 |
| 45 | 40.43±3.98 | 38.50±3.63 | 31.80±2.94 |
| 60 | 40.97±2.73 | 36.90±3.01 | 37.88±2.66 |
| 75 | 39.85±3.51 | 38.85±1.85 | 38.88±5.09 |
| 90 | 40.02±4.23 | 40.80±3.64 | 40.95±1.95 |
| Overall (Mean±SE) | 40.05±1.12 | 38.57±1.04 | 37.82±1.07 |

4.7.3.5. Total Leucocyte Count ($10^3/\mu\text{l}$)

TLC ($10^3/\mu\text{l}$) of different groups have been presented in **Table 4.20**. The overall mean values for different treatment groups were T1 (9.78±0.23), T2 (9.43±0.26) and T3 (9.87±0.34) respectively. No significant ($p>0.05$) difference was observed among different treatment groups over different fortnights.

Table 4.20: Effect of Shatavari root powder on average total leucocyte count ($10^3/\mu\text{l}$) over fortnights

| Days | T1(Control) | T2 | T3 |
|--------------------------|-------------|------------|------------|
| 0 | 10.23±0.54 | 9.60±0.64 | 10.08±1.31 |
| 15 | 9.92±0.40 | 9.53±0.51 | 10.55±0.84 |
| 30 | 9.53±0.48 | 9.27±0.77 | 9.95±0.85 |
| 45 | 9.57±0.62 | 8.80±0.72 | 9.42±1.01 |
| 60 | 10.70±0.46 | 10.08±0.69 | 10.00±1.09 |
| 75 | 8.73±0.94 | 9.65±0.86 | 9.77±0.67 |
| 90 | 9.75±0.61 | 9.10±0.75 | 9.33±0.87 |
| Overall (Mean±SE) | 9.78±0.23 | 9.43±0.26 | 9.87±0.34 |

4.8. Total erythrocyte count ($10^6/\mu\text{l}$)

TEC ($10^6/\mu\text{l}$) of different groups have been presented in **Table 4.21**. The values ranged from to 5.84 to 6.43 in T1 (Control), 5.80 to 6.28 in T2 and 5.79 to 6.22 in T3 group. The overall mean values for different treatment groups were T1 (6.17 ± 0.11), T2 (6.04 ± 0.10) and T3 (6.03 ± 0.09). No significant ($p>0.05$) difference was observed in the T1, T2 and T3 on statistical analysis.

Table 4.21: Effect of Shatavari root powder on average total erythrocyte count ($10^6/\mu\text{l}$) over fortnights

| Days | T1 (Control) | T2 | T3 |
|-------------------------|----------------|----------------|----------------|
| 0 | 6.03 ± 0.29 | 6.02 ± 0.32 | 6.12 ± 0.33 |
| 15 | 6.17 ± 0.35 | 5.99 ± 0.29 | 5.79 ± 0.12 |
| 30 | 5.84 ± 0.21 | 5.80 ± 0.29 | 5.94 ± 0.29 |
| 45 | 6.17 ± 0.31 | 5.96 ± 0.23 | 5.94 ± 0.23 |
| 60 | 6.43 ± 0.40 | 6.23 ± 0.25 | 6.21 ± 0.31 |
| 75 | 6.28 ± 0.37 | 6.28 ± 0.39 | 6.22 ± 0.19 |
| 90 | 6.29 ± 0.18 | 6.04 ± 0.22 | 5.98 ± 0.25 |
| Overall (Mean \pm SE) | 6.17 ± 0.11 | 6.04 ± 0.10 | 6.03 ± 0.09 |

4.9. Beta hydroxy butyric acid (mmol/ L)

The Beta hydroxy butyric acid (mmol/ L) of different experimental groups have been presented in **Table 4.22 & Fig. 4.7**. The overall BHBA was found to be lower ($P<0.05$) in T3 (0.60 ± 0.04) and T2 (0.71 ± 0.04) than control (0.94 ± 0.03).

Table 4.22: Effect of Shatavari root powder on average BHBA concentration (mmol/L) over fortnights

| Days | T1 (Control) | T2 | T3 |
|-------------------------|------------------|------------------|------------------|
| 0 | 1.20 ± 0.05 | 1.13 ± 0.05 | 1.03 ± 0.06 |
| 15 | $1.13^b\pm 0.06$ | $0.96^a\pm 0.04$ | $0.93^a\pm 0.04$ |
| 30 | $1.00^b\pm 0.05$ | $0.85^a\pm 0.04$ | $0.80^a\pm 0.03$ |
| 45 | $0.90^c\pm 0.03$ | $0.68^b\pm 0.06$ | $0.53^a\pm 0.04$ |
| 60 | $0.78^c\pm 0.03$ | $0.58^b\pm 0.06$ | $0.41^a\pm 0.03$ |
| 75 | $0.83^c\pm 0.04$ | $0.46^b\pm 0.04$ | $0.31^a\pm 0.04$ |
| 90 | $0.78^c\pm 0.06$ | $0.35^b\pm 0.04$ | $0.20^a\pm 0.02$ |
| Overall (Mean \pm SE) | $0.94^b\pm 0.03$ | $0.71^a\pm 0.04$ | $0.60^a\pm 0.04$ |

Values (Mean \pm SE) within same row for a particular parameter bearing different superscript differed significantly at $P<0.05$.

4.10. Blood glucose (mg/dL)

The blood glucose concentration (mg/dL) of experimental animals at fortnightly interval have been presented in **Table 4.23 & Fig. 4.8**. Mean blood glucose level at the beginning of experiment was 35.66, 33 and 34.66 mg/dL in Control (T1), T2 and T3 groups, respectively and corresponding values at the end of experiment were 33, 44.33 and 56.83 mg/dL respectively.

Blood glucose level increased with duration of Shatavari supplementation and significant differences in blood glucose were observed in 2nd, 3rd, 4th, 5th and 6th fortnights. Overall mean blood glucose level was significantly ($p < 0.05$) higher in T3 followed by T2 and T1.

Table 4.23: Effect of Shatavari root powder on average glucose concentration (mg/dL) over fortnights

| Days | T1 (Control) | T2 | T3 |
|--------------------------|--------------------------------|--------------------------------|--------------------------------|
| 0 | 35.66 ^a ±0.95 | 33.0 ^a ±1.03 | 34.66 ^a ±1.08 |
| 15 | 34.33 ^a ±1.25 | 35.33 ^a ±1.38 | 37.33 ^a ±0.80 |
| 30 | 34.0 ^a ±1.09 | 37.66 ^{ab} ±1.96 | 40.50 ^b ±0.67 |
| 45 | 34.33 ^a ±1.49 | 39.66 ^b ±1.96 | 43.66 ^b ±1.17 |
| 60 | 33.66 ^a ±0.76 | 41.0 ^b ±1.36 | 47.16 ^c ±1.49 |
| 75 | 33.50 ^a ±0.84 | 42.50 ^b ±1.17 | 50.50 ^c ±1.56 |
| 90 | 33.0 ^a ±0.93 | 44.33 ^b ±0.95 | 56.83 ^c ±2.02 |
| Overall (Mean±SE) | 34.07^a ±0.39 | 39.07^b ±0.77 | 44.38^c ±1.21 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at $P < 0.05$

4.11. Biochemical parameters:

4.11.1. Total protein (g/ dL)

The plasma Protein (g/dL) of different groups have been presented in **Table 4.24**. The values ranged from 4.99 to 5.14 in T1(Control), 5.1 to 5.87 in T2 and 5.22 to 6.27 in T3 group.

The overall value of plasma protein was significantly ($P < 0.05$) higher in T3 group (5.66±0.08) followed by T2 (5.42±0.06) and control (5.07±0.04).

Table 4.24: Effect of Shatavari root powder on average total protein concentration (g/dL) over fortnights

| Days | T1(Control) | T2 | T3 |
|--------------------------|-------------------------|--------------------------|-------------------------|
| 0 | 4.99±0.18 | 5.10±0.17 | 5.22±0.22 |
| 15 | 4.97±0.18 | 5.15±0.16 | 5.27±0.21 |
| 30 | 5.14±0.11 | 5.23±0.15 | 5.41±0.18 |
| 45 | 5.14 ^a ±0.10 | 5.38 ^{ab} ±0.14 | 5.63 ^b ±0.18 |
| 60 | 5.11 ^a ±0.11 | 5.51 ^{ab} ±0.15 | 5.81 ^b ±0.18 |
| 75 | 5.09 ^a ±0.11 | 5.73 ^b ±0.14 | 6.05 ^b ±0.16 |
| 90 | 5.05 ^a ±0.11 | 5.87 ^b ±0.13 | 6.27 ^c ±0.14 |
| Overall (Mean±SE) | 5.07 ^a ±0.04 | 5.42 ^b ±0.06 | 5.66 ^c ±0.08 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at P<0.05

4.11.2. Total albumin (g/dL) in Sahiwal cows:

The albumin concentration (g/dL) of different experimental groups have been presented in **Table 4.25**. The values ranged from 2.8 to 4.2 in T1(Control), 4.1 to 4.9 in T2 and 4.0 to 5.1 in T3 group.

Overall albumin of treatment groups was found significantly (P<0.05) higher than the Control group. The overall mean albumin (g/dL) was significantly higher (P<0.05) in T3 (4.46±0.07) followed by T2 (4.41±0.10) and control (3.40±0.11).

Table 4.25: Effect of Shatavari root powder on average total albumin concentration (g/L) over fortnights

| Days | T1 (Control) | T2 | T3 |
|--------------------------|-------------------------|-------------------------|-------------------------|
| 0 | 4.20±0.18 | 4.10±0.21 | 4.0±0.13 |
| 15 | 3.90±0.30 | 4.10±0.20 | 4.10±0.14 |
| 30 | 3.50±0.30 | 4.20±0.22 | 4.20±0.14 |
| 45 | 3.50 ^a ±0.12 | 4.40 ^b ±0.24 | 4.40 ^b ±0.15 |
| 60 | 3.10 ^a ±0.22 | 4.50 ^b ±0.25 | 4.60 ^b ±0.17 |
| 75 | 2.90 ^a ±0.28 | 4.80 ^b ±0.38 | 4.80 ^b ±0.13 |
| 90 | 2.80 ^a ±0.25 | 4.90 ^b ±0.29 | 5.10 ^b ±0.12 |
| Overall (Mean±SE) | 3.40 ^a ±0.11 | 4.41 ^b ±0.10 | 4.46 ^b ±0.07 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at P<0.05

4.11.3. Cholesterol (mg/ dL):

The Cholesterol concentration (mg/ dL) of different experimental groups have been presented in Table 4.26 & Fig. 4.9. The overall mean cholesterol (mg/dL) was significantly ($P<0.05$) higher in control(T1) (286.62 ± 2.37) than T2 (235.61 ± 5.25) and T3 (228.54 ± 5.58). There was significant difference observed between different treatment group over different fortnights.

Table 4.26: Effect of Shatavari root powder on average cholesterol concentration (mg/dL) over fortnights

| Days | T1 (Control) | T2 | T3 |
|---|---|---|---|
| 0 | 269.17 \pm 4.58 | 270.65 \pm 3.82 | 266.87 \pm 4.58 |
| 15 | 275.07 \pm 5.71 | 266.97 \pm 2.81 | 264.23 \pm 4.32 |
| 30 | 281.43 ^b \pm 5.99 | 257.85 ^a \pm 2.42 | 255.50 ^a \pm 5.25 |
| 45 | 289.00 ^b \pm 3.32 | 243.92 ^a \pm 4.47 | 231.22 ^a \pm 6.83 |
| 60 | 293.9 ^b \pm 4.09 | 222.73 ^a \pm 6.65 | 212.65 ^a \pm 5.96 |
| 75 | 297.62 ^b \pm 4.43 | 202.40 ^a \pm 7.74 | 193.20 ^a \pm 6.80 |
| 90 | 300.13 ^b \pm 4.49 | 184.72 ^a \pm 10.27 | 176.10 ^a \pm 7.31 |
| Overall (Mean\pmSE) | 286.62^b\pm2.37 | 235.61^a\pm5.25 | 228.54^a\pm5.58 |

Values (Mean \pm SE) within same row for a particular parameter bearing different superscript differed significantly at $P<0.05$

4.11.4. Triglycerides (mg/ dL)

The Triglyceride concentration (mg/ dL) of different experimental groups have been presented in Table 4.27 & Fig. 4.10. The overall triglyceride was higher ($P<0.05$) in T1 (58.68 ± 1.93) followed by T2 (45.44 ± 2.01) and T3 (39.14 ± 2.26). The study showed that Shatavari supplementation significantly reduced ($P<0.05$) triglyceride value in treatment groups T2 and T3 than the control.

Table 4.27: Effect of Shatavari root powder on average triglyceride concentration (mg/dL) over fortnights

| Days | T1(Control) | T2 | T3 |
|--------------------------|-------------------------------|-------------------------------|-------------------------------|
| 0 | 61.08±5.18 | 62.74±2.39 | 60.49±5.04 |
| 15 | 57.73±3.86 | 56.15±2.55 | 52.22±4.62 |
| 30 | 60.48 ^b ±6.04 | 51.79 ^{ab} ±2.92 | 43.35 ^a ±2.48 |
| 45 | 57.03 ^b ±5.15 | 45.94 ^{ab} ±2.95 | 35.25 ^a ±2.61 |
| 60 | 57.17 ^b ±6.05 | 38.61 ^b ±3.22 | 32.18 ^a ±2.11 |
| 75 | 58.03 ^b ±6.05 | 34.24 ^b ±2.39 | 27.35 ^a ±2.28 |
| 90 | 59.24 ^b ±5.85 | 28.59 ^b ±2.01 | 23.14 ^a ±2.20 |
| Overall (Mean±SE) | 58.68^c±1.93 | 45.44^b±2.01 | 39.14^a±2.26 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at P<0.05

4.11.5. Blood urea (mg/dL)

The urea concentration (mg/dL) in plasma of different experimental groups have been presented in Table 4.28 & Fig. 4.11. The values (mg/dL) ranged from 24.59 to 27.90 in T1 (Control), 19.63 to 24.08 in T2 and 16.03 to 24.32 in T3 group.

The overall urea (mg/dL) was significantly (P<0.05) lower in T3 (20.78±0.30) and T2 (22.07±0.39) than T1 (25.77±0.24).

Table 4.28: Effect of Shatavari root powder on average blood urea concentration (mg/dL) concentration over fortnights

| Days | T1(Control) | T2 | T3 |
|--------------------------|-------------------------------|-------------------------------|-------------------------------|
| 0 | 24.59±0.50 | 24.08±0.63 | 24.32±0.85 |
| 15 | 24.53±0.43 | 23.77±0.64 | 23.82±0.79 |
| 30 | 25.04±0.58 | 23.22±0.87 | 22.60±0.85 |
| 45 | 25.47 ^b ±0.65 | 21.86 ^a ±0.96 | 20.59 ^a ±1.08 |
| 60 | 26.15 ^b ±0.46 | 21.07 ^a ±1.08 | 19.61 ^a ±1.10 |
| 75 | 26.73 ^b ±0.39 | 20.85 ^a ±1.16 | 18.51 ^a ±1.08 |
| 90 | 27.90 ^b ±0.17 | 19.63 ^a ±0.65 | 16.03 ^a ±1.39 |
| Overall (Mean±SE) | 25.77^b±0.24 | 22.07^a±0.39 | 20.78^a±0.30 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at P<0.05

4.11.6. Creatinine (mg/dL)

The creatinine concentration (mg/dL) of different experimental groups have been presented in **Table 4.29 & Fig. 4.12**. The mean value of creatinine ranged from 1.94 to 2.95 for T1, 1.92 to 2.84 in T2 and 1.82 to 2.24 in T3.

The overall creatinine in T1 (2.41±0.12), T2 (2.34±0.11) and T3 (1.99±0.08) mg/dL. The study showed that Shatavari supplementation reduced creatinine level in treatment groups T2 and T3 significantly (P<0.05) than the T1(control).

Table 4.29: Effect of Shatavari root powder on average creatinine concentration (mg/dL) over fortnights

| Days | T1(Control) | T2 | T3 |
|--------------------------|------------------------------|------------------------------|------------------------------|
| 0 | 2.24±0.28 | 2.45±0.21 | 2.24±0.25 |
| 15 | 2.95 ^b ±.23 | 2.38 ^{ab} ±0.19 | 2.23 ^a ±0.21 |
| 30 | 1.94±0.34 | 2.84±0.37 | 1.99±0.32 |
| 45 | 2.13±0.30 | 1.95±0.26 | 1.63±0.09 |
| 60 | 2.54±.30 | 2.56±0.5 | 2.14±0.33 |
| 75 | 2.81 ^b ±0.38 | 2.32 ^{ab} ±0.31 | 1.82 ^a ±0.15 |
| 90 | 2.23±0.39 | 1.92±0.12 | 1.82±0.17 |
| Overall (Mean±SE) | 2.41^b±0.12 | 2.34^a±0.11 | 1.99^a±0.08 |

Values (Mean±SE) within same row for a particular parameter bearing different superscript differed significantly at P<0.05

4.11.7. Prolactin hormone (µIU/mL)

The prolactin conc. (µIU/mL) of different experimental groups have been presented in **Table 4.30 & Fig. 4.13**. The overall mean prolactin. (µIU/mL) was significantly higher (P<0.05) in T3 (168±5.03) followed by T2 (157±5.61) than T1 (142±3.89).

Table 4.30: Effect of Shatavari root powder on average prolactin hormone concentration ($\mu\text{IU/mL}$) over fortnights

| Days | T1(Control) | T2 | T3 |
|---|--|--|--|
| 0 | 173 \pm 6.47 | 179 \pm 14.11 | 180 \pm 14.11 |
| 15 | 164 \pm 6.90 | 171 \pm 15.59 | 176 \pm 14.13 |
| 30 | 152 \pm 6.76 | 170 \pm 15.56 | 174 \pm 13.87 |
| 45 | 137 \pm 7.52 | 161 \pm 13.03 | 169 \pm 14.14 |
| 60 | 129 \pm 7.34 | 146 \pm 14.93 | 164 \pm 13.78 |
| 75 | 124 \pm 6.84 | 139 \pm 13.41 | 160 \pm 14.29 |
| 90 | 116 ^a \pm 5.70 | 134 ^{ab} \pm 13.19 | 156 ^b \pm 13.11 |
| Overall (Mean\pmSE) | 142^a\pm3.89 | 157^b\pm5.61 | 168^b\pm5.03 |

Values (Mean \pm SE) within same row for a particular parameter bearing different superscript differed significantly at $P<0.05$

4.11.8. Cortisol hormone (ng/mL)

The cortisol conc. (ng/mL) of different experimental groups have been presented in Table 4.31 & Fig. 4.14. The overall mean cortisol (ng/mL) was significantly higher ($P<0.05$) in T1 (**138 \pm 2.89**) followed by T2 (**125.71 \pm 3.58**) and T3 (**138 \pm 2.89**).

There was significant ($P<0.05$) difference observed in the cortisol hormone concentration in different treatment groups over different fortnights.

Table 4.31: Effect of Shatavari root powder on average cortisol hormone concentration (ng/mL) over fortnights

| Days | T1 (Control) | T2 | T3 |
|---|--|---|---|
| 0 | 147.33 \pm 9 | 147.83 \pm 9.45 | 134 \pm 9.27 |
| 15 | 146.33 \pm 8.90 | 142.17 \pm 9.02 | 127.83 \pm 8.32 |
| 30 | 142.50 ^b \pm 8.17 | 131.67 ^{ab} \pm 8.17 | 115.17 ^a \pm 7.03 |
| 45 | 138 ^b \pm 8.57 | 124.50 ^{ab} \pm 7.86 | 106.50 ^a \pm 6.02 |
| 60 | 133.17 ^b \pm 7.25 | 117.5 ^{ab} \pm 6.84 | 98 ^a \pm 5.28 |
| 75 | 129 ^b \pm 6.50 | 111.33 ^a \pm 7.07 | 90.66 ^a \pm 4.01 |
| 90 | 128.83 ^b \pm 3.91 | 105 ^a \pm 6.54 | 84 ^a \pm 2.75 |
| Overall (Mean\pmSE) | 138^c\pm2.89 | 125.71^b\pm3.58 | 108.02^a\pm3.52 |

Values (Mean \pm SE) within same row for a particular parameter bearing different superscript differed significantly at $P<0.05$

Fig. 4.1: Daily Milk yield (litre) per animal per day (week wise)

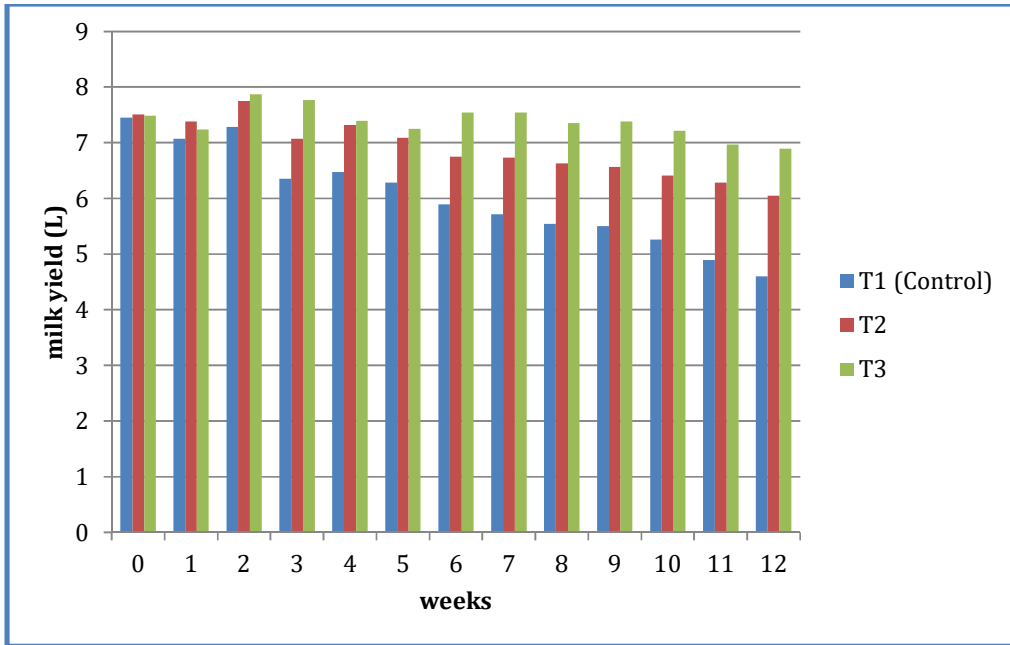


Fig. 4.2: Fortnightly Milk yield (litre) per animal per day

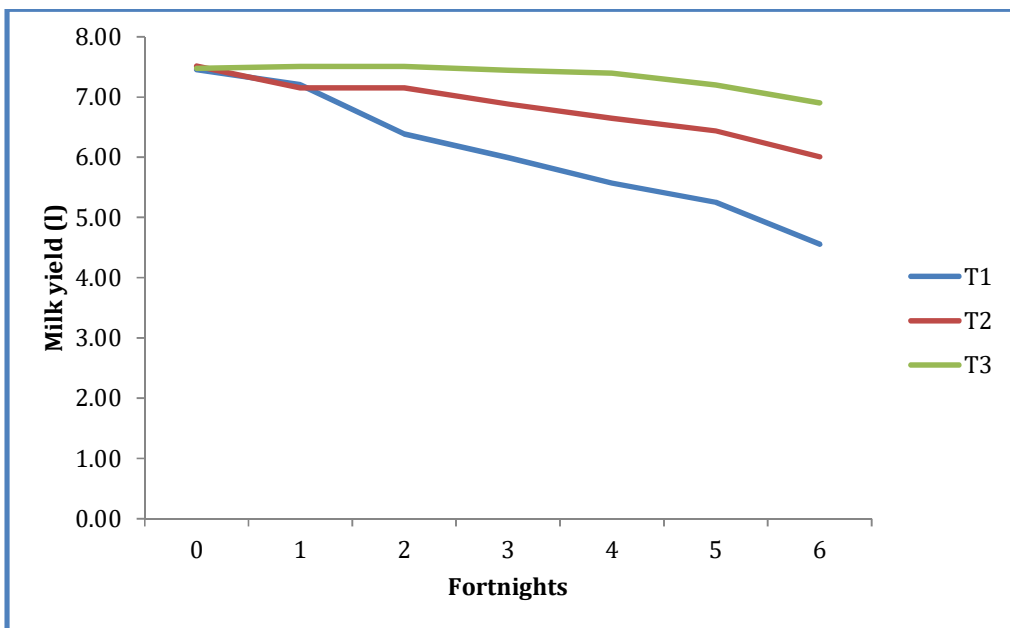


Fig. 4.3: Fortnightly Milk Solid Not Fat (%)

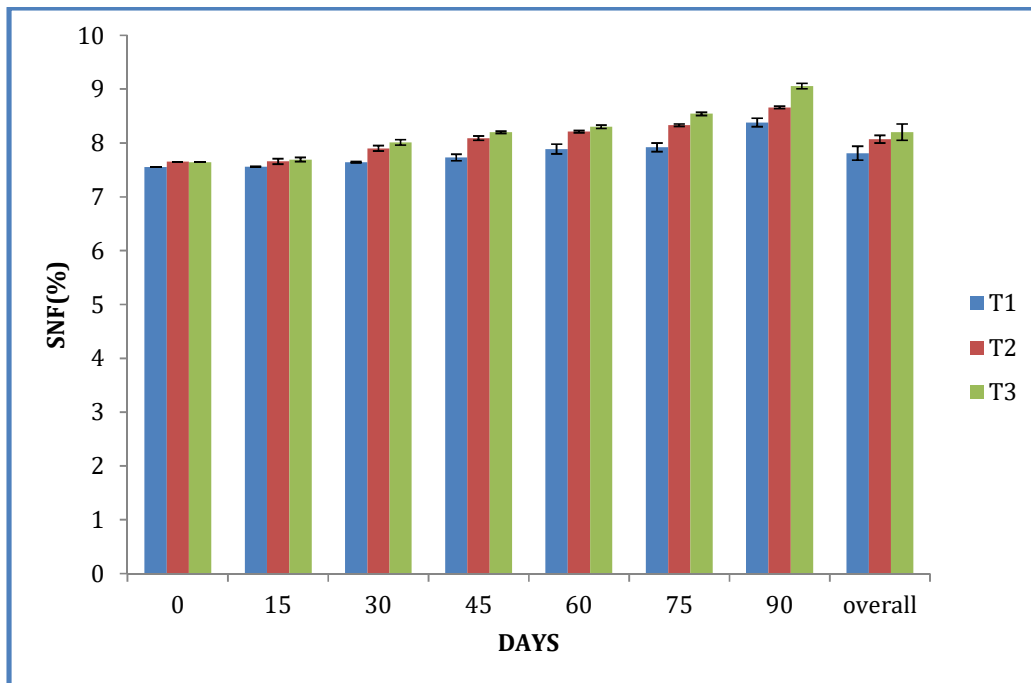


Fig. 4.4: Fortnightly Milk Lactose (%)

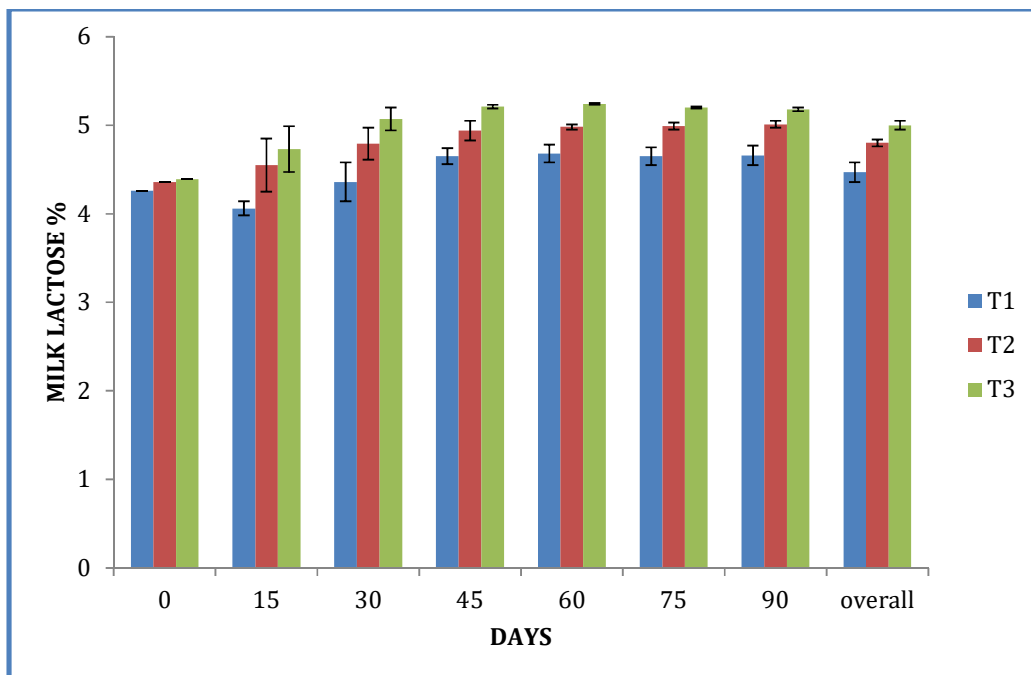


Fig. 4.5: Fortnightly Milk Solids (%)

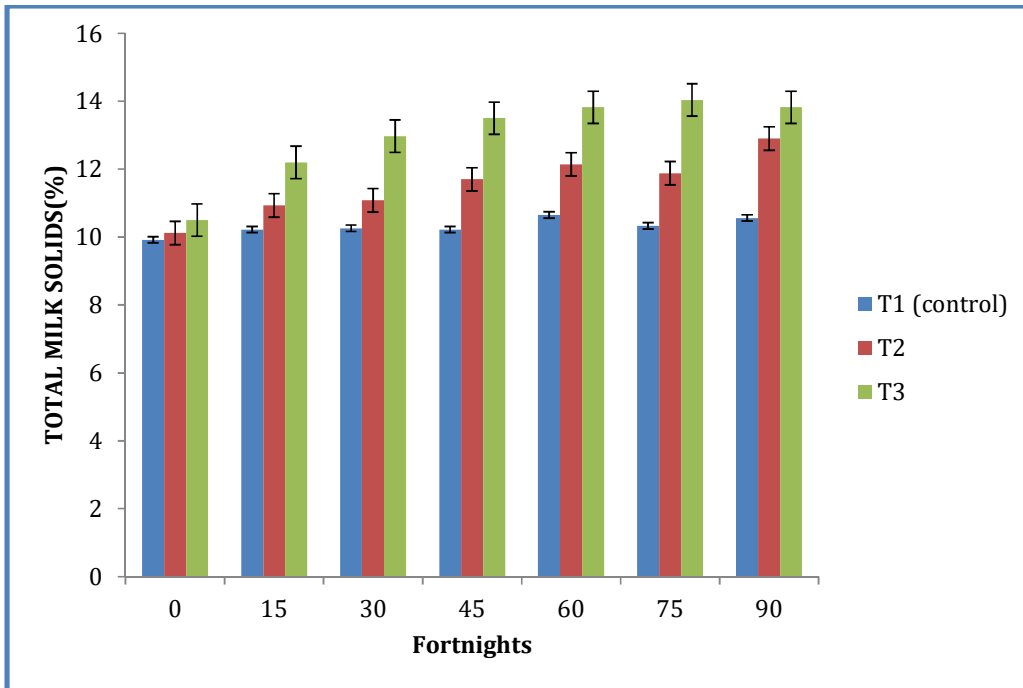


Fig. 4.6: Effect of Shatavari root powder on Body Condition Score

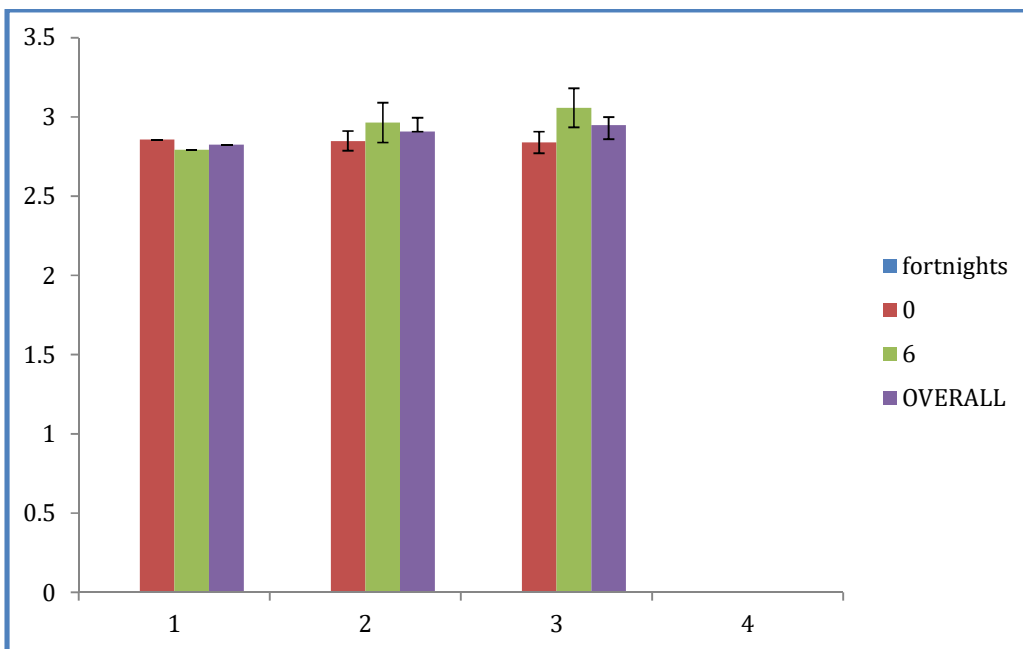


Fig. 4.7: Fornightly BHBA (mmol/L) variation after SRP supplementation

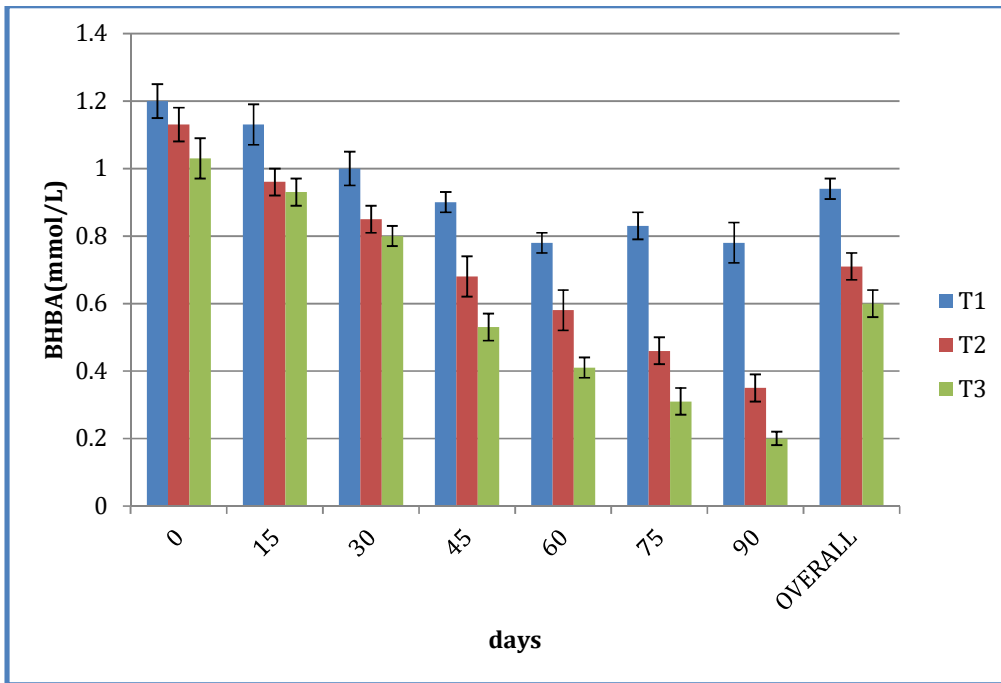


Fig. 4.8: Fornightly blood glucose (mg/dL) variation

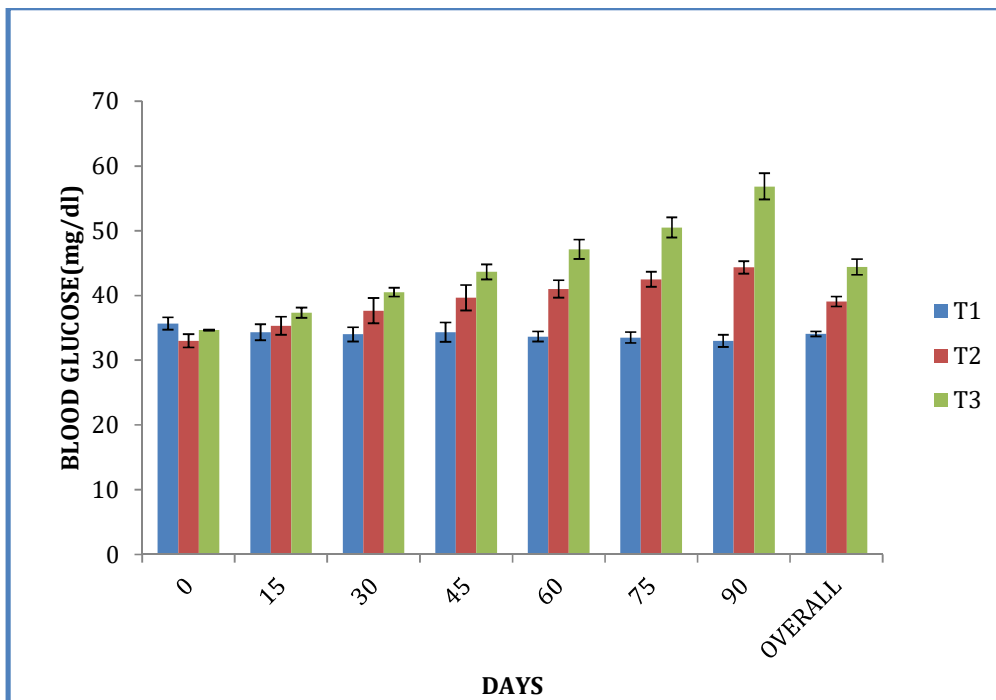


Fig. 4.9: Fortnightly Cholesterol (mg/dL) variation after SRP supplementation

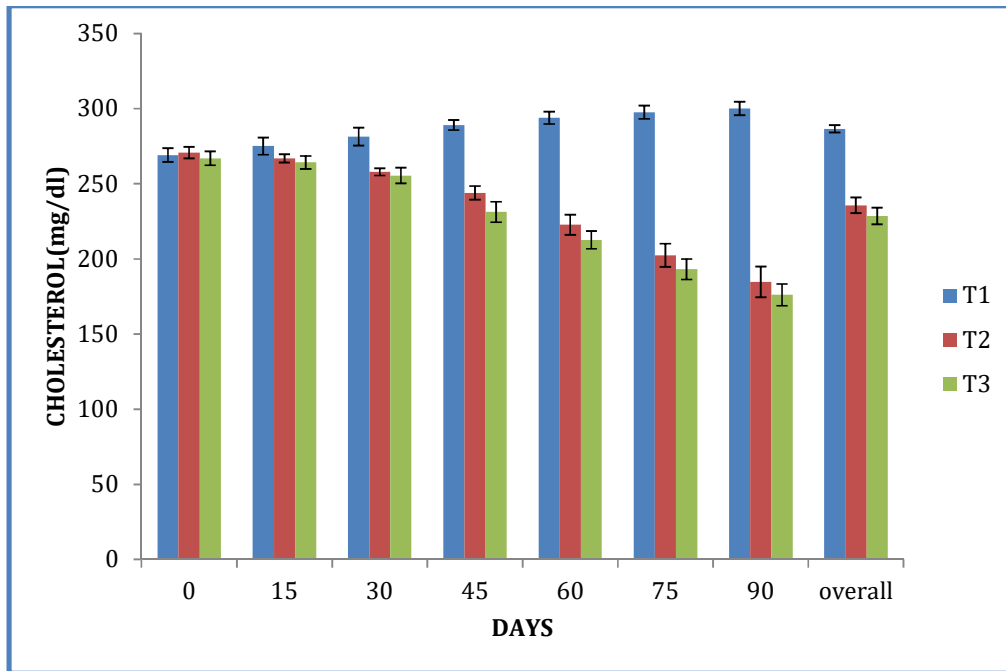


Fig. 4.10: Fortnightly Triglyceride (mg/dL) variation after SRP supplementation

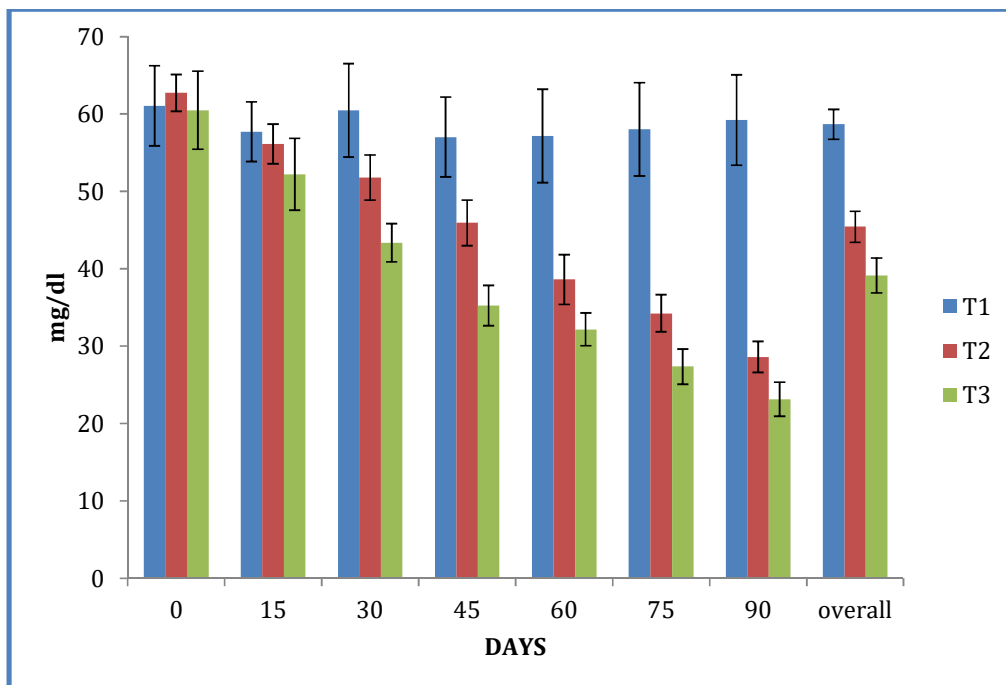


Fig. 4.11: Fortnightly Urea (mg/dL) variation after SRP supplementation

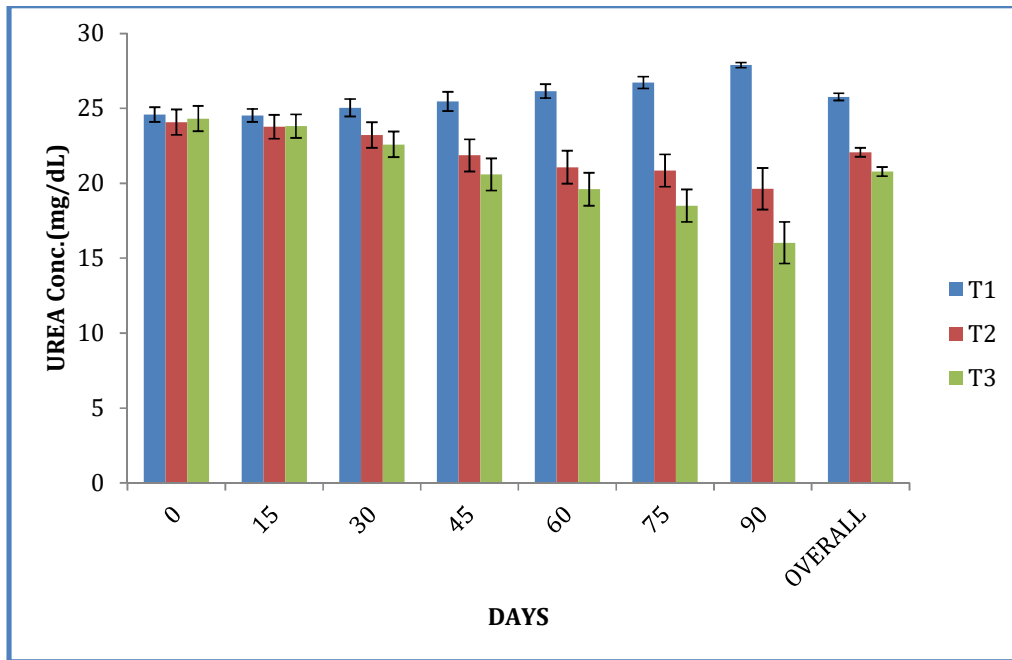


Fig. 4.12: Fortnightly Creatinine (mg/dL) variation after SRP supplementation

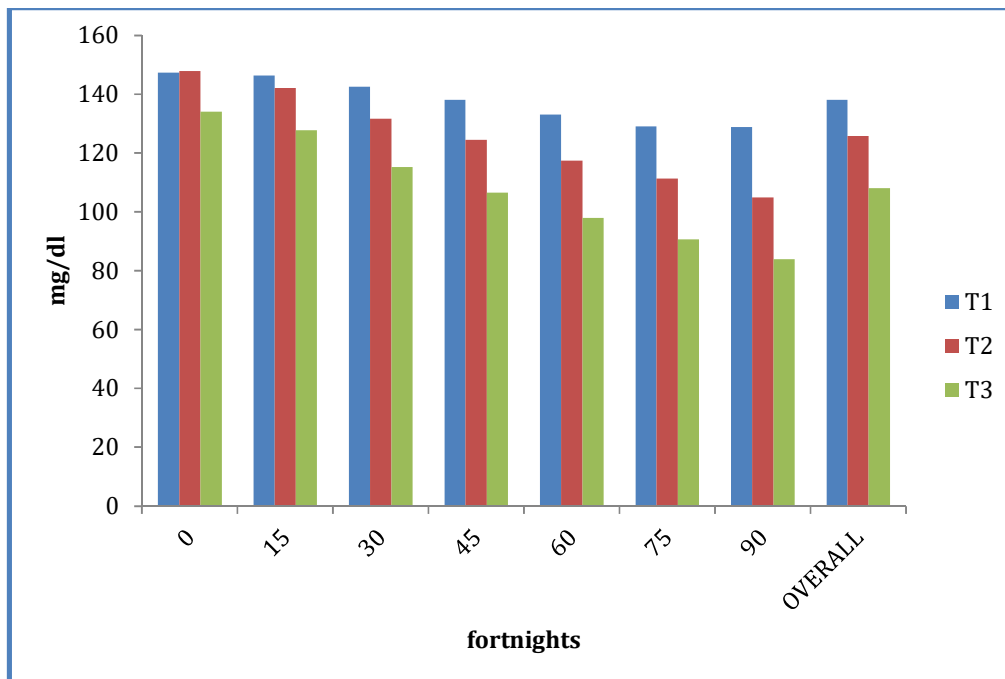


Fig. 4.13: Fortnightly Prolactin hormone ($\mu\text{IU}/\text{mL}$) variation after SRP supplementation

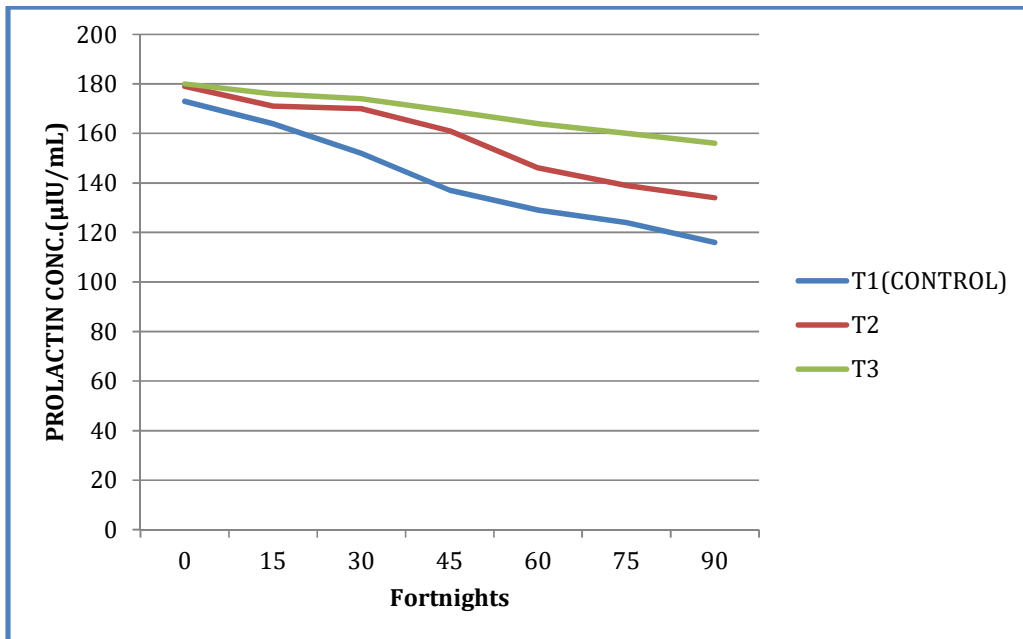
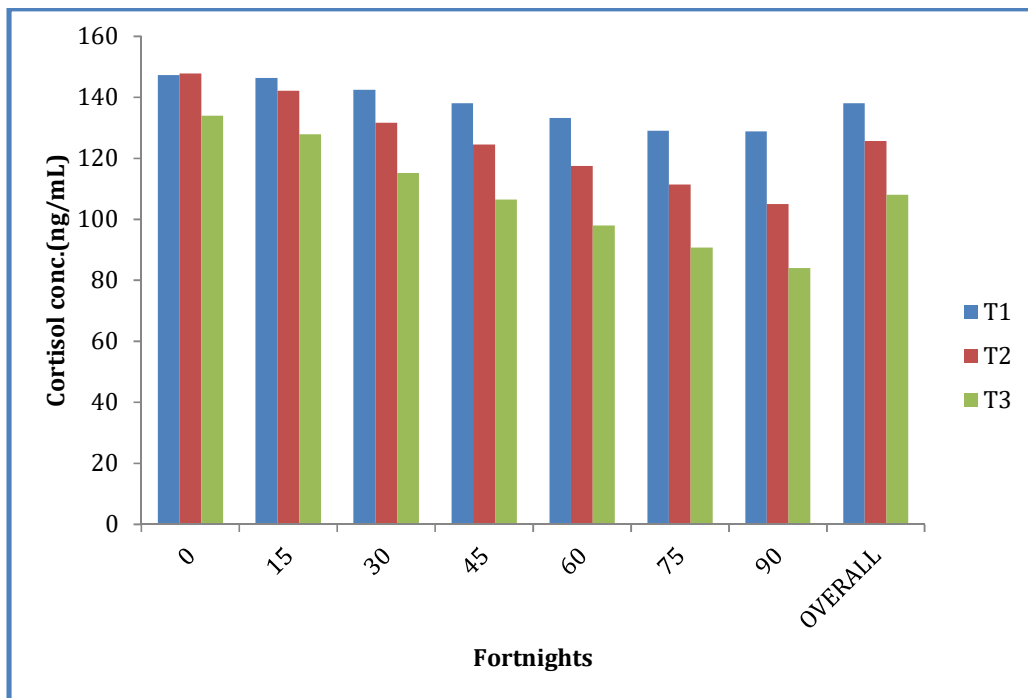


Fig. 4.14: Fortnightly Cortisol hormone (ng/mL) variation after SRP supplementation





Discussion



The results obtained on dietary supplementation of Shatavari root powder on milk yield, milk composition and haemato-biochemical parameters of lactating Sahiwal cows have been discussed in following chapter.

5.1 Chemical composition (% DM basis) of Shatavari root powder

The chemical composition of Shatavari root powder (on dry matter basis) was estimated. The proximate principle i.e., dry matter, crude protein, total ash, crude fibre and ether extract in SRP were found to be 91.3, 4.12, 9.5, 10.58 and 0.82 % respectively.

The findings were in accordance with Berhane and Singh (2000) who reported the DM, CP, EE, CF and Ash Shatavari root powder to be 91.0, 3.85, 0.66, 8.32 and 13.15 % respectively.

Results were also in agreement with Kumari and Gupta (2016), Chavan et al., (2021).

5.2 Daily milk yield (Litre) per cow per day/ Fortnightly milk yield (Litre) per cow per day

The experimental results revealed significant ($P < 0.05$) effect of Shatavari supplementation on milk yield of treatment groups (T1 and T2) compared with the Control group (**Table 4.2 & 4.3**).

These results are in close agreement with the findings of Sharma (2010) who reported significantly ($P < 0.05$) higher milk yield in Shatavari supplemented groups (@200mg/kg) than control group. The increase in milk production by supplementation of Shatavari in crossbred cows could be due to higher levels of the prolactin and insulin hormone in galactagogue herbal mixture (Dadkhah and Yeganehzad. 2011). The results of the present study were almost similar to the findings of Soni et al. (2016) who reported that the milk yield and fat corrected milk yield (FCMY) were found to be significantly ($P < 0.01$) higher in treatment groups supplemented @ 50, 100 and 150 g per day in T1, T2 and T3 than the control group. Patel et al. (2017) also reported that 4% fat corrected milk yield (kg/day) was

significantly higher ($P < 0.05$) in treatment groups (T2@30g, T3 @60 gm and T4 @60 gm) than the control group. The improvement in milk could be due to better udder health and galactopoietic effect of herb in milch animals. According to Sabnis et al., 1968; Ghosh et al., 1987; Singh et al., 2012 Shatavari root powder contains some active components such as steroidal glycosides, saponins, polyphenols, flavonoids and alkaloids which stimulate the hypothalamus or pituitary gland, leading to release of higher levels of prolactin hormone there by increasing the milk production. While estrogenic effect of Shatavari on mammary glands, stimulates alveolar secretory epithelial cell division and proliferation (Sabnis et al., 1968; Pandey et al., 2005) which helps in sustenance of increased milk production.

The results are in accordance with the Ramesh et al. (2000), Mishra et al. (2008), Tanwar et al. (2008), Sharma (2010), Kumar et al. (2011) Dangi (2011), Patel et al. (2013), Kumar et al. (2014), Divya et al. (2015), Soni et al. (2016), Jingar et al. (2018), Saini et al. (2018), Muwal et al. (2020) who also observed significant improvement in milk production performance of dairy animals.

Since , our study was performed in mid lactation stage and in this stage milk production generally declines, but due to Shatavari supplementation it was observed that the rate of decrease in milk yield was very less in the treatment groups compared to control.

5.3 Milk composition

5.3.1. Milk fat (%)

The experimental results revealed no significant ($p > 0.05$) effect of SRP supplementation on milk fat % of experimental group. Overall milk fat % of treatment groups (T2 & T3) were found similar with control group (**Table 4.4**).

These results are in accordance with the findings of Dangi (2011), who reported no significant improvement in fat % of buffaloes supplemented with Shatavari supplementation.

Muwal et al. (2020), Jain and Bais (2015), Berhane & Singh (2000), Berhane and Singh (2002) and Kumar et al. (2014), Bhargav et al. (2021) they also reported non-significant difference in milk fat % after Shatavari supplementation.

However contrary to this, Soni et al. (2016) and Kumawat et al. (2017) observed significantly ($P < 0.05$) higher fat % in Shatavari supplemented group compared to control group. Similarly, Bhinda et al. (2020), Chavan et al. (2021) and Khera et al. (2022) reported significantly higher fat % in Shatavari supplemented group than control group.

5.3.2. Milk Protein (%)

The experimental results revealed no significant ($p > 0.05$) effect of Shatavari root powder supplementation on milk Protein % of experimental animals over the control group (**Table 4.5**). Overall milk protein % of treatment groups was found similar with the Control group.

Similar to present findings Muwal et al. (2020), Bhargav et al. (2021) also reported non-significant ($p > 0.05$) difference in milk protein % of crossbred Sahiwal.

Shatavari being a non- protein source might not cause significant changes in protein content of milk.

However, many previous studies have reported contrasting findings to this as Dangi (2011) in Murrah buffalo, while Kumar et al.(2011), Galbat et al. (2014), Divya et al. (2015) , Saini et al. (2018), Chavan et al. (2021) in crossbred cows, where they reported significant increase in milk protein in Shatavari treatment groups. Wallace et al. (1994), Sen et al. (1998), Abreu et al. (2004), Hess et al. (2004) and Alexander (2005) has explained the positive effect of Shatavari on milk protein is because of saponin and tannin content in shatavari which might have modified the rumen ecosystem. It has also been reported that saponin supplementation reduced rumen ammonia-N concentration and formed complexes of protein that protect protein from degradation in the rumen and finally increased the duodenal flow of microbial-nitrogen. Similarly beneficial effects of low level tannins are increases rumen undegradability of protein and enhances the efficiency of microbial protein production and its assimilation from rumen thus, making more feed protein available post-ruminally for production purposes, which could facilitate sustaining higher milk protein in milk (Wu et al., 1994). To support this explanation, dry matter intake and digestibility studies needed to be done which were lacking in our study.

5.3.3. Milk solid not fat (%)

The experimental results revealed significant ($P<0.05$) effect of Shatavari supplementation on Milk Solid Not Fat % than the Control group **Table 4.6**. Overall milk SNF % of treatment groups T2 ($8.07\pm.05$) and T3 ($8.20\pm.07$) was found significantly higher ($P<0.05$) than Control group.

The results are in agreement with the findings of Kumar et al. (2011), Galbat et al. (2014), Das et al. (2017), Saini et al. (2018), Muwal et al. (2020), and Chavan et al. (2021) as they all reported significant difference in SNF %.

5.3.4. Milk lactose (%)

The experimental results revealed significant ($P<0.05$) effect of Shatavari supplementation on milk lactose % of experimental animals **Table 4.7**. Overall milk lactose % of treatment groups was found significant ($P<0.05$) in the T2 and T3 group than T1 (control). Maximum milk lactose (%) was observed in T3 group followed by T2 group and minimum in T1 group.

Finding of present study are in accordance with Sharma (2010), Kumar et al. (2011), Saini et al. (2018) and Chavan et al. (2021) they all reported significant effect of Shatavari supplementation on milk lactose %.

However findings differed with Kumar et al. (2014) and Muwal et al. (2020), they reported no significant effect of Shatavari supplementation on milk lactose %.

5.3.5. Total milk solids (%)

The experimental results revealed significant ($P<0.05$) effect of Shatavari supplementation on total milk solids % of experimental animals **Table 4.8**. Total milk solid value were significant ($P<0.05$) in the treatment groups than the control.

The findings of current study are in agreement with (Dibya, 2010), who reported the significant ($P>0.05$) effect of Shatavari root powder on total milk solid content in treatment group over different fortnights. Sharma (2010), Galbat et al. (2014), Kumar et al. (2014). Mirzaei et al. (2012), Das et al. (2017), Saini et al. (2018), Bhinda et al. (2020) and Chavan et al. (2021), they also reported significant difference in the milk solid % than the control group. Herbs and their metabolite modify the rumen ecosystem and their supplementation can cause the change in composition of milk.

5.4. Physio-chemical parameters of milk

5.4.1. Milk temperature (°C)

The experimental results revealed no significant ($p>0.05$) effect of Shatavari root powder supplementation on milk temperature. Non-significance difference ($p>0.05$) was observed on milk temperature of T1 (control), T2 and T3 groups.

5.4.2. Milk pH

The experimental results revealed non-significant ($p>0.05$) effect of Shatavari root powder supplementation on milk pH of T1 (control), T2 and T3 groups.

These findings are in agreement with Divya et al. (2015), Bhargav et al. (2021) and Khera et al. (2022), they studied the effect of Shatavari supplementation on pH of milk and found no significant effect ($p>0.05$) of Shatavari supplementation on milk pH.

5.4.3. Density (g/ml) / Specific gravity

The experimental results revealed no significant ($p>0.05$) effect of Shatavari root powder supplementation on density of milk in different treatment groups.

Considering the density of water as 1g/ml the specific gravity of milk remains same as the density of milk. Thus, overall specific gravity of milk was 31.46 ± 0.50 , 31.55 ± 0.55 and 31.68 ± 0.34 for T1 (control), T2 and T3 respectively. Non-significant ($p>0.05$) effect of Shatavari supplementation was observed on specific gravity of milk.

Possible reason for not change in specific gravity could be due to because fat % has not increased while total solids have increased so, specific gravity remain unchanged.

5.5. Somatic cell count ($\times 10^3$ cells/mL)

Overall Milk SCC in the T3 group is 97 ($\times 10^3$ cells/ml) which is significantly lower than T1 (130×10^3 cells/ml). Numerically SCC was also reduced in the T2 group (107×10^3 cells/ml) than the control but the effect was non-significant ($p>0.05$).

Finding of present study are in accordance with Kumar et al. (2014); Dangi (2011), as they also reported significant ($P<0.05$) reduction of milk SCC with Shatavari supplementation in treatment groups.

The Shatavari supplementation reduced number of somatic cell counts in milk, indicating low level of intramammary infection due to Immuno stimulant and Immuno modulatory properties of Shatavari (Rege et al., 1999; Dohoo and Leslie, 1991).

5.6. Body condition score:

The overall mean value of body condition score during the experimental period in T1, T2 and T3 groups are respectively 2.82, 2.90 and 2.94. Non-significant difference ($p>0.05$) was observed between T1 (control), T2 and T3 groups; but numerically T3 had the highest value.

Finding of present study are in confirmity with Patel et al., (2013), they also reported no significant effect of Shatavari on body condition score.

However, contrary to current findings Imtivati (2014) and Kumawat et al. (2017) reported significant ($p>0.05$) increase in body condition score during pre-and post-partum supplementation of Shatavari root powder. The reason they found significant effect might be the part of transition phase (during pre-and post-partum) in their study which itself is a stage of major changes in body condition score. Since our study pertains to mid lactation stage so significant changes were not observed.

5.7. Effect of Shatavari root powder on Haematological parameters

5.7.1. Haemoglobin (g/dL)

Hemoglobin value (g/dl) of different groups have been presented in table 4.14. Non-significant ($p>0.05$) difference was observed in the T1, T2 and T3 but overall numerical value was higher in the T3.

These findings are in accordance with Galbat et al. (2014), who found blood parameters in normal range (10.9-12.8 gm/dL). Results were in disagreement with Shelukar et al., (2001) who reported significant increase in hemoglobin (g/dL).

5.7.2. Effect of Shatavari root powder on other hematological parameters of Sahiwal cows

All the hematological parameters like PCV, DLC, TLC and TEC have been presented in (Table 4.15 to 4.21) results showed non-significant ($p>0.05$) difference in the T1, T2 and T3.

These findings are in agreement with Galbat et al. (2014), who observed no change in hematological parameters after herbal supplementation.

5.7.3. Effect of Shatavari root powder on BHBA (mmol/L) of Sahiwal cows:

The BHBA (mmol/ L) of different experimental groups have been presented in **Table 4.22**. The study shows that the SRP supplemented groups showed significantly lower ketone body concentration than the control.

Plasma BHBA concentration is significantly higher ($P<0.05$) during all the periods in control group as compared to treatment group T3 because of metabolic stress in control group leading to mobilization of body tissue reserves. The intense lipid mobilization probably necessary for the animals to achieve maximal milk yield, but if mobilization is excessive or if normal homeostatic controls break down, metabolic disorders may result (Goff and Horst, 1997). This leads to increased production of BHBA and other ketone bodies which have negative effect on immune system (Klucinski et al., 1988) on the animals.

The higher concentration of BHBA observed in control group might be associated with marked formation of acetyl- coenzyme A, as a result tricarboxylic acid cycle cannot fully metabolize fatty acids (Kunz, et al., 1985). As a consequence, acetyl coenzyme A is converted to acetoacetate which is then reduced to BHBA by BHBA dehydrogenase or spontaneously decarboxylized to acetone (Baird, 1982; Brumby et al., 1975). Hence, the presence of ketone bodies in body fluid is normal to a certain degree, whereas high concentrations of ketone bodies indicate that adaptability of metabolism is exceeded, i.e. that whole body homeostasis cannot be maintained (Aeberhard, et al., 2001 and Baird, 1982).

Earlier reports also indicate that increased BHBA concentration reveals incomplete, oxidation of NEFA in the tricarboxylic acid cycle during negative energy balance (Grummer, 1993 and Doepel et al., 2002).

5.7.4. Effect of Shatavari root powder on blood glucose concentration (mg/dL) of Sahiwal cows:

The blood glucose (mg/dl) of experimental animals at fortnightly interval have been presented in **Table 4.23**. Mean blood glucose level at the beginning of experiment was 35.66, 33 and 34.66 mg/dl in Control (T1), T2 and T3 groups, respectively and

corresponding values at the end of experiment were 33, 44.33 and 56.83 mg/dl respectively.

The significant ($P < 0.05$) increase in blood glucose value of treatment groups T2 and T3 was in accordance with the Galbat et al. (2014), Saini et al., (2018) they also reported significant increase in blood glucose (mg/dl) of Sahiwal supplemented with SRP. The high blood glucose may be due to the glucogenic property of Shatavari. The glucogenic property of Shatavari was also reported by the Berhane and Singh (2000).

Saponin in Shatavari might have increased the propionate production which has generally been considered the major substrate for gluconeogenesis (Wiltout and Satter, 1972), thereby resulting in improved blood glucose level. Alexander (2005) reported that saponin isolated from *Asparagus. adscendens* roots aqueous extract enhanced the production of propionate in growing sheep. Similarly, Hu et al. (2006) and Abreu et al. (2004) also reported that supplementation of saponin in the diet improved ruminal VFA profile and propionate level significantly.

The findings are contrary to the finding of Singh et al. (2012) and Foroozandeh and Ghaffari (2015), they reported non-significant effect of SRP over blood glucose in SRP supplemented groups. However, numerically blood glucose values were higher in the SRP treated groups.

5.8 Effect of Shatavari root powder supplementation on Biochemical parameters:

5.8.1. Total protein (g/dL)

The plasma protein values of different groups have been presented in **Table 4.24**. Results revealed significantly higher total protein in T3 group (5.66 ± 0.08) and T2 (5.42 ± 0.06) than control (5.07 ± 0.04).

These findings are in agreement with Galbat et al. (2014), who reported significant ($p < 0.05$) increase in total protein (g/dL) after Shatavari supplementation in lactating goats.

5.8.2 Total albumin (g/dL)

The albumin value (g/dL) of different experimental groups have been presented in **Table 4.25**. Study revealed that SRP treated groups had significantly ($P<0.05$) higher albumin than the Control group.

Galbat et al. (2014) also observed no change in total albumin (g/dL) after Shatavari supplementation.

5.8.3. Cholesterol concentration (mg/ dL)

The cholesterol (mg/dL) of different experimental groups have been presented in **Table 4.26**.

The study showed that Shatavari supplementation reduced cholesterol in treatment groups T2 and T3 significantly ($P<0.05$).

The present study is in agreement with the findings of Saini et al. (2018) they reported lower cholesterol ($P<0.01$) value in SRP supplemented groups.

However these findings are contrary to the findings of Singh (2010), who reported no effect of Shatavari supplementation on plasma cholesterol (mg/dL). Phyto-sterols are reported to compete and displace cholesterol from the intestinal bile acid micelles and decrease the cholesterol circulation Ikeda et al. (1998) and Quilez J et al., (2003) and on the other hand, saponins precipitate cholesterol from micelles and interfere with entero-hepatic circulation of bile acids making it unavailable for intestinal absorption of cholesterol leading to a reduction in plasma cholesterol levels Harwood et al. (1993) and Oakenfull and Sidhu (1990).

5.8.4 Triglycerides concentration (mg/ dL)

The Triglyceride (mg/dL) of different experimental groups have been presented in **Table 4.27**.

The study indicated that Shatavari supplementation reduced ($P<0.05$) triglyceride value in treatment groups T2 and T3 significantly than the control.

There was significance ($P<0.05$) difference observed in the T2 and T3 groups. Overall triglyceride value was lower in the treatment group 3.

However these findings are contrary to the findings of Singh (2010), who observed no effect of Shatavari supplementation on plasma Triglyceride.

5.8.5. Blood urea concentration (mg/ dL):

The urea concentration of different experimental groups have been presented in **Table 4.28**. Overall mean value for urea is higher ($P<0.05$) in control (T1) (25.77 ± 0.24) than T2 (22.07 ± 0.39) and T3 (20.78 ± 0.30). The study shows that Shatavari supplementation reduced triglyceride value in treatment groups T2 and T3 significantly ($P<0.05$) than the control.

5.8.6. Creatinine concentration (mg/dL)

The creatinine concentration (mg/ dL) of different experimental groups have been presented in **Table 4.29**. The mean value of creatinine ranged from 1.94 to 2.95 for T1, 1.92 to 2.84 in T2 and 1.82 to 2.24 in T3.

The present study revealed that that Shatavari supplementation reduced reduced creatinine level in treatment groups T2 and T3 significantly ($P<0.05$) than the T1 (control).

5.8.7. Prolactin hormone concentration (μ IU/mL):

The prolactin concentration (μ IU/mL) of different experimental groups have been presented in **Table 4.30**. The study revealed that overall prolactin concentration was remained high in Shatavari supplemented significantly ($P<0.05$) treatment groups.

Since our study was done in mid phase of lactation and in mid phase milk yield starts to decline but due to shatavari supplementation prolactin hormone was maintained in quite a good concentration in treatment group T2 and T3.

5.8.8. Cortisol hormone concentration (ng/mL)

The mean value of cortisol for different treatment groups have been presented in the **Table 4.31**.

There was significant ($P<0.05$) difference observed in the mean value of cortisol hormone concentration in the T3 (108.02 ± 3.52) and T2 (125.71 ± 3.58) than the control (138.00 ± 2.89). There was non-significant ($p>0.05$) difference observed in the T2 and T1 but numerically value was higher in T2 than the T1 (control).

Cortisol hormone concentration varied non-significantly during the first two fortnights but plasma cortisol hormone concentration was significantly ($P<0.05$)

higher from third fortnight throughout the experiment in control group as compared to treatment group. The lower cortisol level in treatment group might be due to antistress effect of Shatavari (Wipoonbun et al., 2004). Anti- inflammatory, antioxidant, antistress, antidepressant activity (Satter and Wiltout., 2006).



Summary
and
Conclusions

CHAPTER-6

SUMMARY AND CONCLUSIONS

The hypothesis of present study was to evaluate the effect of Shatavari (*Asparagus racemosus*) supplementation on milk yield, milk composition and haemato – biochemical profile of lactating Sahiwal cows. For this study, 18 lactating Sahiwal cows were selected from the herd maintained at Livestock Farm Complex DUVASU, Mathura. The animals were divided into three groups T1 (Control), T2 and T3 having 6 animals in each group on the basis of their body weight and parity. The animals in control group were maintained on conventional/ standard (basal diet) without any supplementation. T2 and T3 were fed with basal diet along with additional supplementation of Shatavari root powder @ 30 g and 60 g/ animal/day respectively. Nutrient requirement of animals were fulfilled as per ICAR (2013) feeding standards for a period of 90 days. During the experimental period, milk yield per animal was recorded on daily basis for 90 days and milk composition parameters (milk fat, milk protein, milk lactose, SNF, total milk solids), physiochemical parameters of milk (temperature, pH, density and specific gravity), somatic cell count, haematological parameters (haemoglobin, PCV, DLC, TLC, TEC, BHBA/Glucose) were performed at fortnight intervals. Blood samples were collected at 0, 15, 30, 45, 60, 75 and 90 days post supplementation. A fraction of whole blood samples were used for estimating haematological parameters. The remaining amount of blood samples were centrifuged at 3000 r.p.m for 15 min to separate the blood plasma. Blood plasma was used for biochemical parameters (total protein, total albumin, cholesterol, urea/ creatinine, prolactin and cortisol assay).

Effect of supplementation on fortnightly Milk yield per(litre) animal per day

- The overall fortnightly Milk yield per (litre) animal per day (litre) was higher ($P < 0.05$) in T3 (7.35 ± 0.17), T2 (6.83 ± 0.32) than control i.e. T1 ($6.06^a \pm 0.26$).

Effect of Shatavari root powder (SRP) supplementation on Milk composition parameters

- There was no difference ($P > 0.05$) in milk fat percentage T1 i.e. control (3.5%), T2 (3.61%), T3 (3.67%).

- There was no difference ($P>0.05$) in milk protein percentage T1 i.e. control (3.30%), T2 (3.25%), T3 (3.28%).
- Milk lactose % was higher ($P<0.05$) in T3 (5.0%) and T2 (4.80%) than the control i.e. T1 (4.47%).
- Milk SNF % was higher ($P<0.05$) in T3 (8.20%) and T2 (8.07%) than the control i.e. T1 (7.81%).
- Total milk solid % was higher ($P<0.05$) in T3 (12.98 %) and T2 (11.53 %) than the control i.e. T1 (10.31%).

Effect of Shatavari root powder (SRP) supplementation on Physio-chemical parameters of Milk

- There was no difference ($P>0.05$) in milk temperature ($^{\circ}\text{C}$) of T1 i.e. control (35.19 $^{\circ}\text{C}$), T2 (35.21 $^{\circ}\text{C}$), T3 (35.18 $^{\circ}\text{C}$).
- There was no difference ($P>0.05$) in milk pH of T1 i.e. control (7.23), T2 (7.21), T3 (7.23).
- There was no difference ($P>0.05$) in milk Density/specific gravity of T1 i.e. control (31.46), T2 (31.55), T3 (31.68).

Effect of Shatavari root powder (SRP) supplementation on Somatic cell count (cells/ml* 10^3)

- Milk somatic cell count (cells/ml* 10^3) was found to be reduced significantly ($P<0.05$) in T3 (97 cells/ml* 10^3) than T2 (107 cells/mL* 10^3) and control i.e. T1 (130 cells/mL* 10^3).

Effect of Shatavari root powder (SRP) supplementation on Body condition score (BCS):

- There was no significant ($P>0.05$) difference in BCS of T1 i.e. control (2.82), T2 (2.90), T3 (2.94).

Effect of Shatavari root powder (SRP) supplementation on Hemetological parameters:

- There was non-significant ($P>0.05$) difference in Hemoglobin (g/dL) of T1 i.e. control (9.48 g/dL), T2 (9.59g/dL), T3 (9.83g/dL).

- There was non-significant ($P>0.05$) difference in PCV (%) of T1 i.e. control (28.4%), T2 (28.6%), T3 (29.5%).
- There was non-significant ($P>0.05$) difference in DLC (%) of T1 i.e. control (56.16%), T2 (57.94%), T3 (59.11%).
- There was non-significant ($P>0.05$) difference in TEC ($10^6/\mu\text{l}$) of T1 i.e. control ($6.17 \times 10^6/\mu\text{l}$), T2 ($6.04 \times 10^6/\mu\text{l}$) and T3 ($6.03 \times 10^6/\mu\text{l}$).
- There was non-significant ($P>0.05$) difference in TLC ($10^3/\mu\text{l}$) of T1 i.e. control ($9.78 \times 10^3/\mu\text{l}$), T2 ($9.43 \times 10^3/\mu\text{l}$) and T3 ($9.87 \times 10^3/\mu\text{l}$).
- Blood glucose (mg/dL) was significantly ($P<0.05$) higher in T3 (44.38 mg/dL) and T2 (39.07 mg/dL) than the control i.e. T1 (34.07 mg/dL).
- Blood BHBA concentration (mmol/L) was significantly ($P<0.05$) reduced in T3 (0.60 mmol/L) and T2 (0.71 mmol/L) than the T1 i.e. control (0.94 mmol/L).

Effect of Shatavari root powder (SRP) supplementation on Biochemical parameters:

- Total protein (g/dL) was significantly ($P<0.05$) higher in T3 (5.66 g/dL) and T2 (5.42 g/dL) than the control i.e. T1 (5.07g/dL).
- Total albumin(g/dL) was significantly($P<0.05$) higher in T3 (4.46 g/dL) and T2 (4.41 g/dL) than the control i.e. T1 (3.40g/dL).
- Total cholesterol(mg/dL) was significantly ($P<0.05$) reduced in T3 (228.54 mg/dL) and T2 (235.61 mg/dL) than the control i.e. T1 (286.62 mg/dL)
- Triglyceride (mg/dL) was significantly ($P<0.05$) reduced in T3 (39.14 mg/dL) and T2 (45.44 mg/dL) than the control i.e. T1 (58.68 mg/dL)
- Urea (mg/dL) was significantly ($P<0.05$) reduced in T3 (20.78 mg/dL) and T2 (22.07 mg/dL) than the control i.e. T1(25.77 mg/dL)
- Creatinine (mg/dL) was significantly ($P<0.05$) reduced in T3 (1.99 mg/dL) and T2 (2.34 mg/dL) than the control i.e. T1(2.41 mg/dL)
- Prolactin concentration ($\mu\text{IU/mL}$) was significantly ($P<0.05$) higher in T3 (168 $\mu\text{IU/mL}$) and T2 (157 $\mu\text{IU/mL}$) than the control i.e. T1 (142 $\mu\text{IU/mL}$)

- Cortisol concentration (ng/mL) was significantly ($P<0.05$) higher in T3 (108.02) than T2 (125.71) and T1 (138).

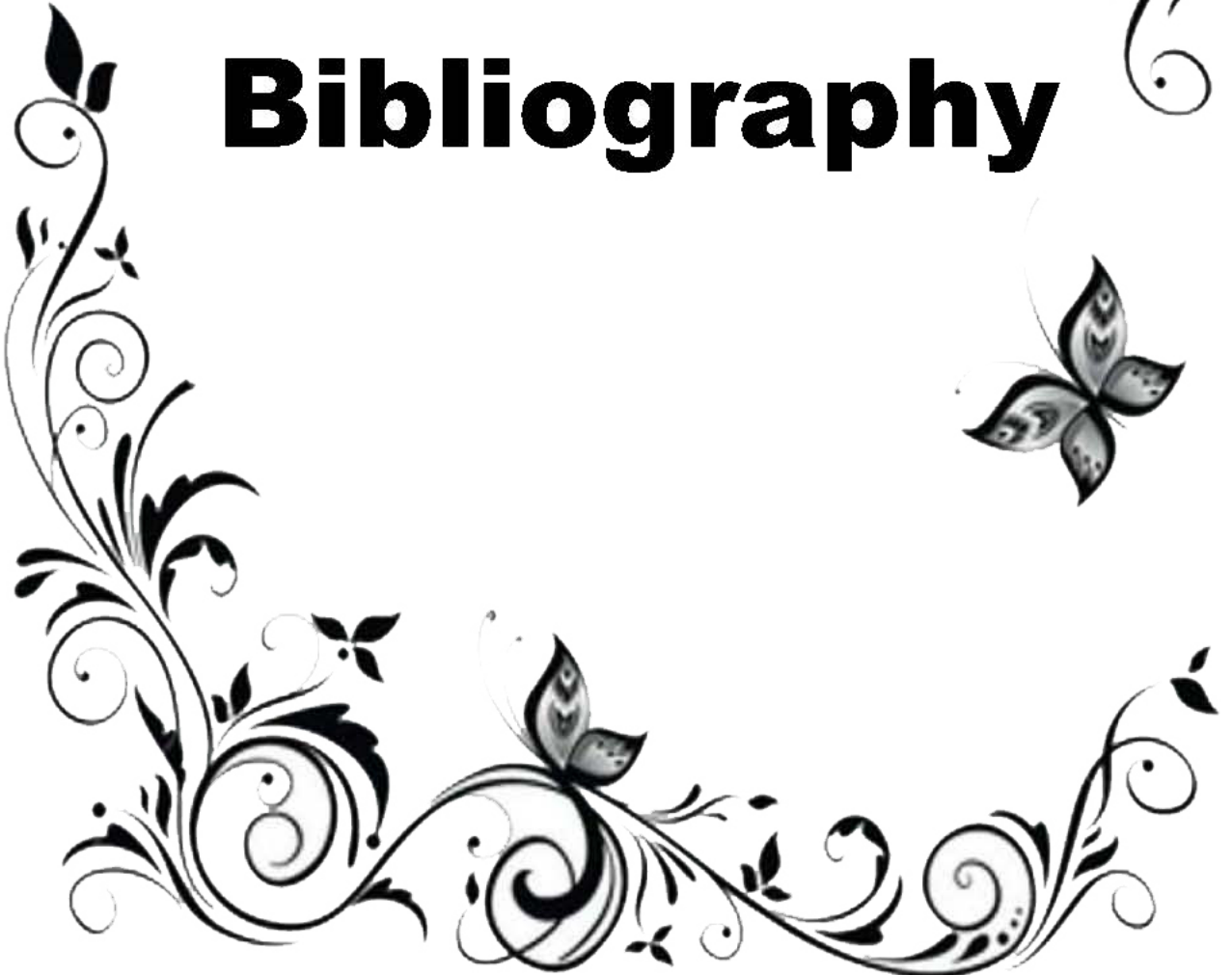
CONCLUSIONS

From the study of effect of shatavari root powder supplementation on the performance of Sahiwal cows, it can be concluded that:

1. Dietary supplementation of Shatavari root powder (SRP) has significant ($P<0.05$) effect on milk yield of lactating cows @60 g.
2. Dietary supplementation of Shatavari root powder (SRP) has significant ($P<0.05$) effect on milk lactose (%), SNF content (%) and total milk solids (%).
3. Dietary supplementation of Shatavari root powder (SRP) has no significant ($P>0.05$) on milk fat(%) and milk protein (%).
4. Dietary supplementation of Shatavari root powder (SRP) has no significant ($P>0.05$) on physio-chemical parameters of milk.
5. Dietary supplementation of Shatavari root powder (SRP) has significant ($P<0.05$) effect on milk somatic cell counts(cells/ml* 10^3).
6. Dietary supplementation of Shatavari root powder (SRP) has no significant ($P>0.05$) on Body condition score.
7. Dietary supplementation of Shatavari root powder (SRP) has no significant ($P>0.05$) on hemoglobin (%), PCV (%), DLC (%), TLC (* 10^3 / μ l) and TEC (* 10^6 / μ l).
8. Dietary supplementation of Shatavari root powder (SRP) has significant ($P<0.05$) effect on blood glucose and BHBA concentration.
9. Dietary supplementation of Shatavari root powder (SRP) has significant ($P<0.05$) effect on total protein, total albumin, cholesterol, triglycerides, urea, prolactin and cortisol hormone.



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Medals/Honours/Fellowships Received

List of Publications

1

1. Effect of Flooring on Behaviour of Haryana calves During Summer Season (poster presentation)

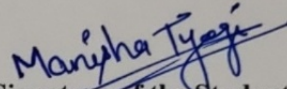
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