EFFECT OF HERBAL FEED SUPPLEMENT SHATAVARI (*Asparagus racemosus*) ON GROWTH AND SEXUAL MATURITY OF SAHIWAL HEIFERS

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

MASTER OF VETERINARY SCIENCE

IN

LIVESTOCK PRODUCTION AND MANAGEMENT

BY

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This is to certify that the thesis entitled, **"Effect of herbal feed supplement Shatavari (*Asparagus racemosus*) on growth and sexual maturity of Sahiwal heifers "** submitted by **Dr. MOHABBAT SINGH JAMRA** in partial fulfilment of the requirement for award of the degree of **MASTER OF VETERINARY SCIENCE** in **LIVESTOCK PRODUCTION AND MANAGEMENT** of the National Dairy Research Institute (Deemed University), Karnal (Haryana), is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

Date: 23 **June, 2012**

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MAJOR ADVISOR GUIDE

Dedicated to Guide, Parents And My Wife

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(Mohabbat Singh Jamra)

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ABBREVIATIONS	
A .racemosus	: *Asparagus racemosus*
ACTH	: Adrenocorticotropic Hormone
ADF	: Acid Detergent Fiber
ADG	: Average Daily Gain
ANOVA	: Analysis of Variance
Av	: Average
BW	: Body weight
CP	: Crude Protein
Dec.	: December
DM	: Dry Matter
DMI	: Dry Matter Intake
EE	: Ether Extract
ELISA	: Enzyme Linked Immunosorbant Assay
Feb.	: February
g	: Gram
G1	: Group 1
G2	: Group 2
GH	: Growth Hormone
GR	: Glucocorticoids Receptor
Jan.	: January

kg	: Kilogram
mg	: Milligram
ml	: Millilitre
MR	: Mineralocorticoid Receptor
NDF	: Neutral Detergent Fiber
NDRI	: National Dairy Research Institute
ng	: Nanogram
Nov.	: November
NRC	: National Research Council
Oct.	: October
OM	: Organic Matter
SE	: Standard error
Sept.	: September
Vs.	: Verses
@	: At the rate of
%	: Percent
µL	: Microlitre

ABSTRACT

There are 166.10 million Indigenous cattle in India among them heifers are 15.75 million (18th Livestock census, 2010). The Indigenous cow contributes 43 % of total milk production. Feeding, housing and other management needs constantly change from birth to first calving of heifer. Heifer's growth should be monitored for multiple reasons: to avoid delays in sexual maturity and first calving due to slow growth to determine whether heifers are overfed or underfed, to get "ideal" body weight at first calving, thereby minimizing calving interval. Shatavari is found to be having phytoestrogenic, immunostimulant, immunomodulator, anabolic, antistress, galactagogue and mammogenic properties. Feeding of shatavari have also resulted in enhanced estrus activity and improved conception rates in cattle. Keeping in view all these properties of *shatavari* and the problems with indigenous cattle performance the present study was formulated and effect of Shatavari was observed on growth rate and sexual maturity of Sahiwal heifers. A total of 16 heifers were selected and divided into two groups on basis of body weight and age. Shatavari was fed to treatment group @ 150 mg/kg b.wt./animal/day over and above NDRI schedule whereas control group was fed according NDRI feeding schedule only. All the parameters were tested for difference between treatment and control group at 5 % level of significance. There was significantly higher overall growth rate in treatment group than control group from 5th fortnight. Dry matter intake (DMI) in treatment group was slightly higher than control group after 5th fortnight and also significantly higher body weight was observed in treatment group after 7th fortnight. Cortisol levels not differed significantly in the two experimental groups. There was significantly higher overall growth hormone level from 7th fortnight onwards (except 9th fortnight) in treatment group compared to control group. Puberty was attained significantly early in treatment group (713.60±16.10 days) than control group (739.66±19.17 days). In treatment group there was significantly lower age at first service (817.40±20.37) than control group (846.10±24.0). Feed supplementation of *Shatavari* @ 150 mg/ kg live body weight / day for six months improved body weight, growth rate, dry matter intake and level growth hormone while supplementation of Shatavari lowered age at puberty and age at first services in Sahiwal heifers.

सारांश

भारत में 166.10 मिलियन देशी गाय हैं उनमें बछिया 15.75 मिलियन हैं । (18 वी पशुधन गणना, 2010 तक) तथापि, प्रति दिन औसतन पैदावार इन पशुओं की बहुत कम हैं ।कुल दूध उत्पादन का 43 प्रतिसत देशी गाय से मिलता है । साहिवाल बछिया की अच्छी विकास दर अच्छी प्रबंधन का संकेतक है । जन्म और पहला प्रसव के बीच खान-पान, आवास एवं अन्य प्रबंधन जरूरतों मे निरंतर बदलाव आते है । बछिया के विकास की निगरानी निम्न कारणों से रखनी चाहिए: यौन परिपक्वता में देरी और प्रथम बार प्रजनन विकास की धीमी गति के कारण से बचने के लिए । बछिया के अल्पपोषण या अधिक्पोषण को निर्धारित करने के लिए । प्रथम प्रजनन के बाद बछड़ा के शरीर के आदर्श वजन के लिए । सतावरी में फायटोएस्ट्रोजेनिक, प्रतिरक्षा उत्तेजक, प्रतिरक्षा न्यूनाधिक, उपचय, तनाव विरोधी, गलक्टोगोग और मम्मो जेनिक गुण पाए गए हैं । सतावरी खिलाने के परिणामस्वरूप मद गतिविधि और गर्भाधान दरों में सुधार पाया गया है देशी गाय में उपर्युक्त समस्याओं को ध्यान में रखते हुए वर्तमान अध्ययन तैयार किया गया था और साहिवाल गाय में सतावरी का विकास दर और लैंगिक परिपक्वता पर प्रभाव देखा गया । कुल 16 बछिया चुने गए थे और शरीर के वजन और आयु के आधार पर दो समूहों में विभक्त किया गया था । प्रायौगिक समूहों को सतावरी 150 मि. ग्रा./कि. ग्रा. शरीर के भार /दिन एन डी आर आइ सारणी से अधिक खिलाया गया और नियंत्रण समूहों को एन डी आर आइ सारणी के तहत खिलाया गया । कुल विकास दर 5 वे पखवाड़ा के बाद प्रायौगिक समूहों में नियंत्रण समूहों की तुलना में महत्वपूर्ण रूप से अधिक पाई गयी ($P < 0.05$) । डी एम आइ 5 वे पखवाड़ा के बाद प्रायौगिक समूहों में नियंत्रण समूहों की तुलना में थोड़ी अधिक पाई गयी ($P < 0.05$) । जबकी डी एम आइ 7 वे पखवाड़ा के बाद प्रायौगिक समूहों में नियंत्रण समूहों की तुलना में महत्वपूर्ण रूप से अधिक पाई गयी ($P < 0.05$) । कोर्टिसोल के स्तर में दोनों समूहों में कोई महत्वपूर्ण अंतर नहीं देखा गया ($P < 0.05$) । ग्रोथ हार्मोन का स्तर 7 वे पखवाड़ा के बाद (केवल 9 वे पखवाड़ा को छोड़कर) प्रायौगिक समूहों में नियंत्रण समूहों की तुलना में महत्वपूर्ण रूप से अधिक पाया गया ($P < 0.05$) । तरुण अवस्था प्रायौगिक समूहों में नियंत्रण समूहों की तुलना में महत्वपूर्ण रूप से जल्दी आ गयी थी (713.60±16.10 vs. 739.66±19.17 दिन ($P < 0.05$) । प्रायौगिक समूहों में प्रथम सेवा (ऐ आई) नियंत्रण समूहों की तुलना में महत्वपूर्ण रूप से कम आयु में आ गयी थी । तरुण अवस्था (वयस्क शरीर के भार के 50 प्रतिसत) और लैंगिक परिपक्वता (वयस्क शरीर के भार के 60 प्रतिसत) में शारीरिक वजन में महत्वपूर्ण रूप से शीघ्र आयु में आ गयी थी ।

CHAPTER – 1

Introduction

1.0 INTRODUCTION

The role of livestock sector is very crucial in the economy of India. The increasing in human population and their shift toward dairy products as a diet is causing rise in demand for high quality dairy food and thus is putting pressure on dairy sector. The indigenous cattle population is 166 million (18th livestock census 2007) and among those heifers are 15.75 million. There is decrease of 0.8 % in heifer population compared to 15.87 million in 17th livestock census 2003. Heifers are future herd of a dairy farm. Heifers are required to replace 20 -25 % of the older and uneconomical females of the farm through voluntary culling, (Etgen and Reaves, 2002). The growth rate and age at maturity are directly linked with productive and reproductive performance of a female. The early age at maturity and growth of heifer will produce calve early, increase numbers of calf and last longer in the milking herd, complete more lactation and thus will produce more milk in her lifetime. Heifer growth rate and body weight at sexual maturity is of an extreme importance in dairy farm management (Sejrsen and Purup, 2000). The increased growth rate in heifers can decrease the duration of non-productive state (Sejrsen and Purup, 2000). Lower growth rate in early life of heifer results in higher age at puberty and thus higher age at first calving and lifetime productivity of these animals is low resulting into late maturity and light weight at the onset of production, long dry period and calving interval (Jabbar *et al.,* 2000). Average age at first calving in Sahiwal cattle is 46 months which is much higher than that of Holstein cows being 29 months (Bashir, 2006; Rehman, 2006). Age at puberty and calving is related with weight (Moore *et al*., 1990). Heifers can be bred when they have attained 60% of their adult body weight (Hammond J., 1960). The feed and management program for replacement heifers will have a lifelong effect on their productivity. Heifer growth rate is an indicator of management level. Feeding, housing and other management needs are constantly changing between birth and first calving.The variability in the growth rate of heifers may reflect that seasonal availability of forages (quantity and quality) and management decisions to adjust heifer growth to a desired rate. The birth body weight of Sahiwal calves is 20 to 25kg in male and 18 to 23 kg in female. Adult body weight is around 540 kg in males and 327 kg (range 301 to 360 kg) in females.

Sahiwal is well known dairy cattle breed in the tropical and subtropical regions of world for its excellent heat and tick resistance. The value of adequate nutrition and management of replacement heifers is mostly overlooked and production losses linked with slow growth rate are not entirely realized.

Age at puberty is an important determinant of reproductive efficiency. Many heifers, especially taurine, can reach puberty and breed fairly satisfactorily at one year old. However, the cost of achieving this varies among breeds and among heifers within the same breed. Heifers with the inborn ability to reach puberty early thus attain puberty and breed at less cost than those with later inherent age at puberty. Estimates of age at puberty in *Bos indicus* cattle in the tropics and subtropics range between 16 and 40 months (Ahuja et al 1961; McDowell et al,1976; Malik and Ghei, 1977 and Aria and Cristofori,1980). *Bos indicus* cattle reach puberty later than *Bos taurus* x *Bos indicus* crossbreeds or purebred taurine cattle (McDowell *et al,,* 1976; Aria and Cristofori,1980). This is due to genetic and environmental factors, including nutrition, disease, temperature and season of birth. These factors affect heifer growth rates.

In India, the improvement in dairy cattle is the difficult due to uneducated rural masses, supplementary source of income, low productivity and scarcity of land. The significance of adequate nutrition and management of replacement heifers is often ignored and production losses linked with slow growth rate are not realized. Animal productivity can be increased up to 40% just by manipulating the macro and micro nutrients with existing gene pool (Sarwar, 2010). Protein and energy are most critical nutrients influencing animal productive performance under tropical/subtropical environment conditions (Shahzad *et al*., 2010). If the supply of protein is adequate, then dietary energy is major limiting factor for ruminant growth and protein supplement alone to low energy diet has no effect on growth rate (Mtenga and Madsen, 1992). Poor nutrition delays puberty, reduces conception rate and increases pregnancy losses in heifers (Lemenager et al, 1980). The future of any dairy operation depends upon a successful program for raising heifers which equal or exceed the current level of milk production. Unfortunately, this is the most neglected area because during this period heifers are not generating income directly as well as require feeding, housing and veterinary expenses with no visible returns.

Ayurveda is the originated from India and oldest health care discipline practiced for past 4000 to 5000 years. The ancient history of India is very rich in

herbal medicine and one of the oldest surviving system of health care in the world and known as *Ayurveda* derived from its ancient Sanskrit roots '*Ayur*' (life) and '*ved*' (knowledge) it offers a rich, comprehensive outlook to a healthy life. Its essence across the globe and has occupied a prime position in health care systems despite more advance in modern medicine system which is chemical based. *Ayurveda* is a natural remedy and totally based on herbs. These herbs are being used since Pre-Vedic times because they are safe to use, cheap and easily available, have no side effect and no residual effect in milk (Krishna *et al.,* 2005). There are several herbs which have been described in the *Ayurveda* to improve the general well beings, milk production and reproduction of both human and animals. *Ayurveda* also described some name of herbs such as *Withania somnifera* (Ashwagangha), *Leptadenia reticulata* (Jivanti), *Lipidium sativum* (Chandasoor), *Alternanthera sessilia* (Kanchari), *Trigonela foenumgrasum L.* (Maithi), *Asparagus racemosus* (Shatavari) etc.

Shatavari (Asparagus racemosus) is an important medicinal plant of tropical and subtropical India. The genus *Asparagus* includes about 300 species around the world. Out of the 22 species of *Asparagus* recorded in India. *Shatavari* is a woody climber growing 1- 2 m in height, the root are finger-like and clustered. The leaves are like pine needles, small and uniform and the inflorescence has tiny white flowers in small spikes. This plant belong to *Liliaceae* family, is common at low altitudes in shade and in tropical climates throughout India, Asia, Australia and Africa. In Sanskrit, the meaning of *shatavari* is described as 'able to have one hundred husbands and in *Ayurveda* this amazing herb is known as the "queen of herbs" because it promotes love and devotion and as it increases the capacity for lovemaking (Simon, 1997). According to *Ayurveda* some important action of *shatavari* are that it increases strength of the body (*Balya*), stabilise the age i.e. delays the effects of old age on the body and mind (*Vayah sthapana*), rejuvenates the body (Rasayana), increases the memory and the analytical ability of a person (*Medha agni pushtida*), rejuvenates the eyes (Netrya), useful in management of diarrheal conditions (*Atisarajit*), increases the quality and quantity of sperms in semen (*Shukrakari*), improves the functioning of mammary glands (*Stanyakari*). It is also useful in management of all edematous conditions (*Shothajit*), cardiac tonic (*Hrudya*), increases sexual desire (*Vrishya*), useful in management of hemorrhoids and assimilatory disorders (*Nihanti arsho grahni*), useful in conditions related with emaciation and under nutrition (*Kshyapaha*), great aphrodisiac and rejuvenator of

the reproductive system (*Shataveerya*), one having hundreds of sons (*Bahuuta*) and having very good nourishing quality (*Atirasa*).

There is several use of *shatavari* for human being but, there is no report on effect of *shatavari* supplementation on growth and sexual maturity in Sahiwal heifers. To fill up this vacuum in knowledge of Sahiwal heifers the present study is proposed with the following objectives:

1. To study the effect of herbal feed supplement Shatavari on growth performance of Sahiwal heifers.

2. To study the effect of herbal feed supplement Shatavari on sexual maturity of Sahiwal heifers.

CHAPTER – 2

Review of Literature

2.0 REVIEW OF LITERATURE

The heifers are future cow of the herd. The productivity performance of cows depends on the management and nutrition given to them during their heifer hood. It is generally recognized that indigenous dairy heifers have lower growth rates throughout life than crossbreds. The heifers raised to use consistent, high quality nutrition and management. The heifers have tremendous changes during the growing period upto their sexual maturity. The importance of this period in determining health, productivity and profitability has been underscored by the intense interest in nutrition and management of dairy heifer during the transition period over the last several years. Heifer growth rate is an indicator of management level. Feeding, housing and other management needs are constantly changing between birth and first calving. Heifer growth should be monitored for multiple reasons to avoid delays in sexual maturity and first calving due to slow growth to determine whether heifers are overfed or underfed to get ideal body weight at first calving. There is an urgent need to develop a holistic approach which will not only fulfill single problem but will improve overall performance of dairy heifer. Such approaches have been described in the Vedic scripture '*Ayurveda*'. *Ayurveda* described that *shatavari* overcomes all reproduction related complications and improves overall physiological system so called pillars of health including digestion, energy metabolism, immune and reproductive system.

The literature available on *shatavari* (*Asparagus racemosus*) supplementation and its effect on digestion, immune system, milk production, udder and reproduction health, energy metabolism and blood metabolites of dairy cows has been reviewed as per the objectives of the study under the following heads-

2.1 Chemical composition of *Shatavari*

Chemical composition of *Shatavari* feed supplement has been presented in Table (2.1).

Table 2.1: Plant secondary metabolites and proximate composition of *Shatavari* herbal feed supplement

Name of Phytochemicals	% Content (%DM basis)
Total Phenolics	4.57
Total Tannin	3.68
OM	90.7
DM	93.2
CP	6.47
EE	0.35
Total Ash	2.50
Acid sol. Ash	0.43
NDF	38.14
ADF	13.40
Hemicellulose	24.74

(Berhane, 2000; Mishra *et al*., 2005).

2.1.1 Chemical constituents of *Shatavari*

Shatavari (Asparagus racemosus) have a rich source of steroidal saponins have been reported in the roots in which shatavarins I and shatavarins IV (Jadhav and Bhutani,2006). shatavarin V, racemosides (Mandal et al.,2006) tapogenins and saponin, from various parts of the plant, (Oketch-Rabah, 1998; Lacaille-Dubois, 2000). The presence of saponin in shatavari root has been reported (Jadhav and Bhutani, 2006). Beside saponin, root extract of shatavari contain flavonoids (6.7±3.9 mg/100ml), polyphenol including tannin (88.2±9.3 mg/100ml) and Vitamin-C (42.4 ± 5.1 mg/100ml) (Velavan *et al*., 2007). Mishra *et al.* (2005) reported that shatavari root contains 4.6 to 6.1% protein, carbohydrates 36.8 to 47.5%, phenols 3.1 to

5.2mg/g, tannins 4.8 to 5.1 mg/g, saponin 4.1% and ash 6.5 to 7.4%. The presence of phyto-components in *shatavari* root such as phytosterols (0.79%), saponin (8.833%), polyphenols (1.692%), flavonoids (0.476%) and total ascorbic acid (0.762%) were also estimated by Visavadiya and Narasimhacharya, (2007). Kamat and Venkatachalam (2004) reported that shatavari root extract has different types of polysaccharide components (Mol. Wt. 2000 kilodaltons) such as galactose (54%), glucose (28%), Rhamnose (4%), xylose (5%) and arabinose (8%) and others (1%).

2.1.2 Effect of chemical constituents of *shatavari* on ruminant nutrition

The literatures which are available on effect of *shatavari* on feed intake in dairy animals are with respect to DM intake. However, there is paucity of documented literature on the effect of chemical constituents present in *shatavari* on rumen parameters. Saponin and tannin, present in *shatavari* are well explored in ruminant nutrition; the effect of saponin and tannin in ruminant nutrition are reviewed in the proceeding sections.

2.1.2.1 Effect of Saponin in ruminant

Till recently, animal nutritionists considered saponin to be deleterious compounds. However, now beneficial effects of saponin on ruminants have been reported (Sen *et al.,* 1998a; Malik, 2006).

2.1.2.2 Effect of Saponin on nutrient digestibility

Wang *et al.* (2000b) reported that saponin extract of *Yucca schidigera* enhanced the fermentation of high grain diet; however, no such effect was observed with alfalfa hay which indicated that action of saponin was diet dependent. Digestibility of DM, OM and CP was improved following the supplementation of diet with fenugreek seed (500 mg/kg) in Zaraibi goats (Allam *et al*., 1999) and this effect was attributed to the saponin content of fenugreek seed. Killeen *et al*. (1998) proposed that a surfactant or flocculent action of the saponin from *Yucca schidigera* altered the rate and site of CP digestion, which accounted for the substrate dependent nature of saponin.

In a recent study, Abreu *et al*. (2004) reported that total tract NDF digestibility was not affected by the dietary supplementation of *Sapindus saponaria* in sheep. NDF digestibility was not affected in the rumen or in total tract in sheep following alfalfa root saponin supplementation @ 1-4 per cent of DMI (Klita *et al*., 1996). NDF

degradability was not influenced by the incorporation of saponin Yucca powder (Hristov *et al.*,1999) or *Enterolobium cyclocarpum* (Hess *et al*., 2003b), however, it was reduced by 12-15 per cent on incorporating *Pithecellobium saman* (200mg/kg) and *Sapindus saponaria* (100 mg/kg) in the diet of sheep.

The positive effects of saponin are more pronounced when they are directly administered into the rumen rather than added to the feed. Wang *et al*. (2000b) observed that supplementation with *Yucca* extracts might be beneficial to ruminants fed a high-grain diet. *Yucca* saponin were found to have a direct negative effect on cellulolytic bacteria while being harmless to amylolytic bacteria, suggesting the possibility of using saponin for 'designing' the rumen population.

Alexander (2005) did not observe any deleterious effect of plant extract supplementation of different herbs and medicinal plants on apparent DM digestibility.

2.1.2.1.3 Effect of Saponin on rumen ammonia nitrogen

A positive effect of Yucca saponin in ruminant nutrition was attributed to the enhancement of the entrapment of NH_3-N from urea-supplemented straw. This increases the availability of nutrients to rumen bacteria and reduces environmental damage by decreasing losses of NH_3 to the air. Supplementation of feed with leaves of *Sesbania sesban*, known for its high saponin content, has been found to have the potential to improve protein flow from the rumen by suppressing protozoal action there but rumen bacteria were observed to be capable of metabolizing the anti-protozoal factor (Makkar *et al*., 1999).

Inclusion of sarsaponin in the diet of heifers decreased ruminal ammonia concentration significantly (Hristov *et al*., 1999). Lila *et al*. (2003) reported that ammonia nitrogen decreased in an *in vitro* experiment following the supplementation of diet with saponin. Headon *et al*. (1991) hypothesized that glycol-component of Yucca extract bound with ammonia and saponin fraction may affect ammonia concentration indirectly via their toxicity to rumen ciliate protozoa.

Extract of *Y. schidigera* plant (a saponin-rich plants, as a herbal medicine) has been found to improve growth, feed efficiency and health in ruminants (Mader and Brumm, 1987). Quillaja saponin increased the efficiency of *in vitro* rumen-microbial protein synthesis and decreased degradability of feed protein (Makkar and Becker, 1996). Partially hydrolyzed lucerne saponin administered intra-ruminally resulted in a

significant reduction in the total protozoa count in the rumen of sheep (Lu and Jorgensen, 1987), which may be the reason for the decrease in feed protein degradability. Saponin is considered to have detrimental effects on protozoa through their binding with sterols present on the protozoal surface. Sterols are absent on bacterial membranes. *Yucca* extract can also bind NH_3 when ruminal NH_3 concentrations are high, and release it again when ruminal NH_3 is low, providing a continuous and adequate supply of NH_3 for microbial protein synthesis.

Similarly, Lu and Jorgensen (1987) reported that addition of pure saponin at 5 per cent level (on DM basis) to the diet reduced ammonia nitrogen in rumen significantly. Alexander (2005) supplemented the substrate with plant extracts of *Acorus calamus*, *Asparagus adscendens* and *E. quisetum*, which are all rich in saponin and found that the supplementation reduced the ammonia-N concentration in the incubation medium, irrespective of the type of extraction solvent when these were used at higher concentration (0.625 mg/ml). At lower concentration the effect was not significant. In spite of higher saponin content in *E. quisetum* extract, it did not affect the ammonia nitrogen concentration even at higher level. Therefore, it was concluded that the supplementation of plant extract not necessarily affect the ammonia nitrogen concentration during rumen fermentation.

Abreu *et al*. (2004) and Hess *et al*. (2004) found an increase in duodenal flow of microbial-nitrogen in sheep fed *Sapindus saponaria* fruit. However, Hristov *et al*. (1999) did not obtain a significant effect of Yucca saponin on microbial protein flow to the intestine in heifers. An increase of microbial nitrogen supply, efficiency of microbial-nitrogen supply, and faecal-nitrogen excretion with increasing levels of *Sapindus rarak* extract was observed, but this increase was not significant. Saponin has also been evaluated with a view to enhance protection of protein from degradation in the rumen and increase availability of protein post-ruminally. Inhibition of rumen proteolytic activity of Yucca saponin has been demonstrated (Wallace *et al*., 1994). Saponin also form complexes with proteins and could decrease protein degradability (Sen *et al*., 1998b). *Sapindus rarak* saponin did not affect the protein degradation in *in vitro* rumen system (Muetzel *et al*., 2005). On the other hand, Quillaja saponin decreased protein degradability of the concentrate, but not of hay-based diet (Makkar and Becker, 2000). These observations suggest that the nature of diet plays a considerable role in determining the effects of saponin. The addition of

S. saponaria fruit to a sheep diet decreased plasma urea, suggesting that less ammonia was absorbed from the rumen (Abreu *et al*., 2004; Hess *et al*., 2004).

2.1.2.1.4 Effect of Saponin on rumen TVFA production

TVFA concentration is an index of microbial digestion of diets. Malik (2006) reported that TVFA concentration increased significantly on increasing the level of berseem and lucerne fodders or their extracts in the diet. Recently, Abreu *et al*. (2004) reported that steroid saponin and triterpenoid saponin increased the propionate level and decreased the butyrate level.

Alexander (2005) reported that addition of *A.* adsendens extract (aqueous) to the *in vitro* system increased the propionic and butyric acids concentration in the incubation medium without affecting the concentration of acetic acid and efficiency of microbial protein synthesis. Wang *et al*. (2000b) also reported a decrease in acetate and an increase in propionate production on various diets incorporated with legume green fodder or pure saponin.

2.1.2.1.5 Effect of Saponin on rumen microbes

Shatavari root extract have been reported to have antimicrobial property against different types of pathogenic bacteria, helminthes, virus, fungi and protozoa (Bhatnagar *et al*., 1961; Swarup and Sharma, 1967; Singh *and* Sharma, 1978; Renu, 1983; Ahmed *et al*., 1998; Perumal *et al*., 1998; Mandal *et al*. 2000). However, reports are available only on pathogenic organisms. The literature on the effect of shatavari on rumen organisms is not available.

Shatavari contains steroid saponin. Steroid saponin has been found to be detrimental to several infectious protozoans. The toxicity of saponin to protozoa seems to be widespread and non-specific and is obviously the result of their detergent effect on the cell membranes. The antiprotozoal property of saponin may be lost upon deglycosylation (Wang *et al*., 2000a).

The protozoa suppressing effect of *Sapindus saponaria* fruits has been demonstrated *in vitro* (Hess *et al.,* 2003b) and *in vivo* (Diaz *et al.,* 1993). Abreu *et al*. (2004) reported that supplementation of *Sapindus saponaria* fruits to the diet consisting of tropical grass with and without legume increased the protozoal counts by 67% in the rumen liquor of the sheep. Patra *et al.* (2006) reported that inclusion of *A. concinna* and *A. indica* extracts in the medium resulted in a significant reduction in

protozoal count. Decreased protozoal counts with supplementation of saponin rich extract (Hristov *et al*., 1999) or saponin rich forages (Newbold *et al*., 1997; Teferedegne, 2000) or fruits (Thalib *et al*., 1996; Hess *et al*., 2003a) have also been reported. Saponin possibly binds with sterol of cell membrane of protozoa and changes the permeability of cell membrane. Adverse effects of extracts of *A. indica* on total, small and large entodiniomorphid protozoa might be due to the presence of bitter principles. Malik (2006) investigated the effect of saponin containing forage plant (extracts of berseem/ lucerne *in vitro* as well as *in vivo*) and found that the compound has a potential to decrease protozoal numbers.

It can be inferred from the review that saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body. However, there is a paucity of information on the saponin containing medicinal plant such as *shatavari* (*Asparagus racemosus*) which is being extensively used in *Ayurveda* in India and their influence on the production performances in crossbred cattle.

2.2.1 Effect of tannin on ruminant nutrition and production

Tannins are polyphenolic substances. Their multiple phenolic hydroxyl groups lead to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids and polysaccharides. These are considered to have both adverse and beneficial effects depending on their concentration and nature as well as other factors such as animal species, physiological state of the animal and composition of the diet. Some of the beneficial effects of tannins are enhancement of rumen undegradable protein and making feed protein available post-ruminally for production purposes, enhancement of efficiency of microbial protein production and protection of ruminants from bloat. Some tannin is also known to have strong anticarcinogenic and antioxidant activities (Perchellet *et al*., 1996; Riedl *et al*., 2002; Makkar *et al.*, 2007).

Higher concentration of tannins reduces the feed intake and digestibility by decreasing palatability of the ration because of its astringent effect on oral cavity. Astringent sensation arises due to binding of tannin with salivary glyco-proteins, which impart lubricant property to mucin (Glick and Joslyn, 1970; Waghorn *et al.*, 1994; Barman, 2004; Barman and Rai, 2006; Dubey, 2007). However, lower

concentration of tannin in the diet increased milk production and growth rate in cows and calves which was attributed to the protection of dietary protein from degradation in the rumen (Bhatta *et al*., 2000; Dubey, 2007). Diet low in tannin increase efficiency of microbial protein synthesis (Makkar *et al*., 1995a and b), decreasing the protein degradability of feed protein in the rumen and passing nitrogen to the lower intestine which could improve the milk and milk quality in term of protein percent (Waghorn *et al*., 1994a; Wang *et al*., 1996; Harris *et al.*, 1998 and Woodward *et al.*, 1999). Some evidence exists for lower protozoal number (Wang *et al*., 1994) and higher molar proportion of propionate (Waghorn *et al*., 1994, Makkar *et al*., 1995a and b) in presence of tannin.

2.2.1.1 Health benefits of Shatavari Phytochemicals

Type and quantity of Phytochemicals which are found in shatavari have been reviewed under section 2.26. Phytochemicals constitute an important part of dietary intake of human and animals and in the past decade, the bioactivities of Phytochemicals on human health have given rise to much attention. So, the health benefits of these Phytochemicals are being reviewed in brief.

2.2.1.2 Health benefits of polyphenols

Several thousand different polyphenols exist in the plant kingdom which can be divided into different subclasses. The main subclasses that are important from a heath perspective are the flavanes, flavonols, isoflavanes, and flavanones. Scientific data from epidemiological studies suggest that diets high in flavonoids potentially reduce the risk of chronic disease. Beneficial effects of polyphenol (tannin) in dairy animals are dependent on the chemical and physical structure, and concentration in the diet. They have been shown to improve live weight gain, milk yield and milk protein concentration, and ovulation rate. They prevent bloat in cattle and reduce gastrointestinal nematode numbers and fly strike (Waghorn and Warren, 2003).

2.2.1.3 Health benefits of flavonals

The common flavonols including kaempferol myricetin and quercetin are found in *shatavari*. Most of the pharmacological effects of quercetin are ascribed in part to its antioxidant activity (Zhang, 2005), anti-inflammatory, antiulcer, and antiviral activities (Brown, 1980; Middleton and Kandaswami, 1992; Rajbhandari *et al*., 2001).

2.2.1.4 Health benefits of isoflavones

Isoflavones also called phytooestrogen are flavonoids and to date are the most important phytochemical in women heath and found to reduce hormonal disbalance (Zhang, 2005). Supplementation of phytoestrogen rich feed increased milk production, milk fat, milk protein, and milk lactose (Dewhurst *et al*., 2003) and contents of phytoestrogens in milk may be important when the health benefits of milk are studied (Steinshamn *et al*., 2008) but it can impair fertility as reported in sheep (Austin *et al*., 1982).

2.3 1 Effect of Shatavari on feed intake and digestibility

Shatavari is an important traditional medicine and used in digestion related problems. As a traditional medicine, shatavari is effective in diarrhoea, dysentery, pain (Roy *et al*., 1971; Nadkarni, 1976; Singh and Ali*,* 1994), dyspepsia and indigestion (Dalvi *et al.,* 1990). Shatavari is a digestive tonic and it prevents hepatic disorders and repair gastric lesion (Dahanukar *et al.,* 2000; Muruganadan *et al.,* 2000; Sairam *et al.,* 2003; Bhatnagar *et al*., 2005; Dharmani and Gautam, 2006). Shatavari reduced the gastric empting time, antimicrobial properties against pathogenic bacteria and protozoa, amylase, lipase and cholinesterase enzymes activities are responsible to improve the digestion and digestion related complications in animals (Dalvi *et al.,* 1990; Vijaya and Vasudevan, 1994; Gupta and Gupta, 1997; Mandal *et al.,* 2000a; Nair *and* Chanda, 2006).

Herbal feed supplements are known for their beneficial effect on rumen ecosystem. Herbal feed supplements have different type of plant secondary metabolites. Low concentration of plant secondary metabolites such as tannin and saponin improves microbial protein synthesis and reduces protozoa count and consequently reduces the degradability of protein and NH_3 concentration in rumen. The herbal feed supplements are also known to improve feed intake through increasing the digestibility of feed (Kumar *et al*., 2006). Shatavari is a well documented digestive tonic and appetizer (Dalvi *et al*., 1990) and feeding a herbal formulation containing 25% shatavari enhanced dry matter intake significantly by 10.97% in buffaloes (Mahantra *et al*., 2003), and overcome the indigestion problems *and* reduced the protozoan counts in one-year calves (Pradhan, 1995).

Postpartum *shatavari* supplementation in lactating crossbred cows at the rate of 100g on alternate day (Barhane *and* Singh, 2002) and 100g/day/animal (Mishra *et al*., 2008) have been found to increase DMI significantly. The increase in DMI might be due to improved digestibility of nutrients (*In Vitro)* (Bakshi *et al.,* 2004; Wadhwa and Bakshi, 2006; Kumar *et al.*, 2006). The digestibility of feed was further increased when shatavari was used in combination with other herbs (Bakshi *et al.,* 2004; Kumar *et al.*, 2006).

2.4.1 Effect of herbal feed supplement on anabolic action

Sharma *et al.* (1986) supplemented root extract of shatavari in a dose of 100 mg/kg BW for a varying period of 4 week to 8 months and observed growth promoting effects in pregnant rats. Shatavari treated animals also showed a better weight gain 81.19 % as compared to the control animals 67.9 %. There was no any side effect observed on the progeny of treated animals.

Gupta *et al*. (2004) supplemented *shatavari* with the combination of *Cryptolepis buchanani* at the rate of 1% of DM as feed additive. The herbal feed supplement leads to significant improvement in digestibility of feed nutrients consequently significant higher body weight gain in supplemented female calves as compared to un-supplemented group.

2.5.1 *Shatavari* as an anti-oxidant, anti-stress and anti-depressant

Dhingra and Kumar (2007) investigated the effect of *shatavari* on depression in mice and results concluded that shatavari has significant antidepressant property. The efficacies of the extracts were found to be comparable to fluoxetine and imipramine.

Parihar and Hemnani (2004) also found that shatavari is effective against free radical mediated diseases and it preventes from oxidative stress.

Visavadiya and Narasimhacharya (2005), Vaidya and Devasagayam, (2007) and Velavan *et al*. (2007) reported that aqueous extract of shatavari scavenged superoxide anion radicals, hydroxyl radical, nitric oxide radical, and hydrogen peroxide significantly. The antioxidant property may be related to the presence of antioxidant vitamins C, micronutrients and phenolic or racemofuran compound (Wiboonpun *et al.*, 2004). Kamat *et al*., (2000) also revealed the antioxidant properties of shatavari against damage induced by gamma-radiation in rat liver

mitochondria. The role of anti-oxidant and anti-stressor in dairy cow especially the periparturient period is critical for health and subsequent performance.

Indeed, parturition and the immediate periparturient period impose conditions of extreme stress on the dairy cow which leads to oxidative stress and can endanger the animal health (Guidry *et al*., 1976; Nakao and Grunert, 1990; Prabhakar *et al*., 1999). On the contrary of oxidative stress, dietary supplementation of antioxidant in the diet of pregnant and lactating cows reduced the incidences of intramammary infection, mastitis, SCC, retention of placenta and improved reproductive performance (Thomas *et al*., 1990; Thomas *et al*., 1992, Gerloff, 1992; Panda, 2003, Panda and Kaur, 2008, Khan, 2008). Therefore antioxidant and anti-stress agent supplementation has profound importance in dairy cow's overall performance.

It has been reviewed that shatavari is a natural and well proved antioxidant; however, the role of shatavari as an antioxidant in dairy animal is not evaluated so far. However, supplementation of herbal antioxidant in dairy animal may be beneficial as human because requirement of antioxidant in dairy animals are found more during lactation and pregnancy and low concentration of antioxidant resulted in oxidative stress to the animals and consequently more susceptible to diseases (Weiss, 1998).

2.6.1 Effect of *Shatavari* on reproductive performance

Reproductive performance is the determinant of reproductive health and is evaluated in cows in terms of calving interval, number of services/ inseminations per conception, days open/service period and reproductive disorders. Prepartum administration of immunopotentiators and antioxidant appears to be beneficial, promising and offer improvements to postpartum reproductive performance (Panda, 2003; Sattar *et al*., 2003b; Sattar *et al*., 2003c and Sattar *et al*., 2007). Non-specific immunostimulants have received considerable attention in dairy farming. They appear to provide an efficient way of stimulating the reproductive performance in a non-specific manner with few adverse side effects. However, very little information is available regarding herbal immunopotentiator supplementation during both prepartum and postpartum period or alone and its effect on reproductive performance of dairy animals.

In Vedic scripture the beneficial effect of herbs on reproduction are well mentioned. In *Ayurveda*, *shatavari* has been described as absolutely safe for long term use, even during pregnancy and lactation. Supplementation of *shatavari* is recommended during last and first trimester of pregnancy to restore the mother's energy, boosts the immunity of both mother and fetus, promote quality of milk and to ease *vata* and promote digestion (*Maasaanumaasika Pathya, Garbhini Paricharya, Prasuthi Tantra Ayurveda*). For newly mothers *shatavari* is useful for boosting the immune system of both the mother and fetus and considered the best herb for balancing hormone levels and strengthening the reproductive system. It is also described as substances beneficial for maintenance of pregnancy (*Garbhasthaapaka Aushadhi* / *Garbha sthaapaka dravyas*), counter act the factors responsible for abortion (*garbhopaghathakara bhaavas*) and help in the proper maintenance of the fetus (*garbha*) *i.e.* prevention from abortion. *Shatavari* can be used as a routine as they are beneficial for the maintenance of proper health, growth and development of the mother and fetus.

Shatavari is mentioned under six important *Rasayana* in *Ayurveda* (Goel and Sairam, 2002) and considered an important herb for women overall health and vitality and as an aphrodisiac. *Shatavari* for tones, cleanses, nourishes and strengthens the female reproductive organs and it nourishes the ovum and increases fertility. So it is traditionally used for PMS and sexual debility (Frawley, 1989), ammenorrhea, dysmenorrhea, dysfunctional uterine bleeding (Swarup and Umadevi, 1998; Chopra and Simon, 2000), menopause and pelvic inflammatory disease like endometriosis (Hemprabha *et al*., 2001; Prasad *et al*., 2002) and gonorrhea (Thomsen, 2002). It also supports deeper tissue and builds blood and thus helps to remove infertility, prepare the womb for conception, prevents miscarriage and acts as a postpartum tonic where it helps to increase lactation and normalize the uterus after parturition, prolapse of uterus and the changing hormones (Tirtha, 1998).

In *Ayurveda shatavari* has been described as best herb for women but scientific studies on the effect of *shatavari* supplementation on reproductive performance in cows is scanty. In a study by Berhane, (2000) reported that supplementation of *shatavari* (100g on alternate day) postpartum alone led to 100% estrus and 75% conception in treatment group as compared to 50% in control crossbred cow within 90 days of calving (table no. 2.2).

Table 2.2 Effect of shatavari on reproductive performance on prepartum and postpartum of cross bred

Sl.No	Treatments					
	Control			Shatavari supplementation		
	BW (Kg)	Reproductive status		BW (Kg)	Reproductive status	
		Estrus	Pregnancy		Estrus	Pregnancy
1	290	*	*	240	*	*
2	350	*	*	270	*	*
3	280			310	*	*
4	330			370	*	

Hedge *et al*. (2002) conducted a trial with 5 dairy cows in each group. The treated group was fed with @ 100g shatavari root powder + 10 gm Aloe dried pulp powder per animal/day and control group was fed only by Aloe 10gm / animal after A.I. for 10 days and reported that 60 % animals were conceived in treatment group however, 100% animals were repeated in the control group.

2.6.2 Effect of herbal feed supplement *Shatavari* on sexual maturity

The reproductive performance of dairy cattle is a major concern in both organized and unorganized dairy farming and is declining continuously (Luck, 2001and McDougall, 2006); therefore, excellent herd management is required for maintaining high milk production as well as high fertility (Leroy, 2006). To improve the overall productivity, Prepartum and postpartum management play vital roles, but most of the interventions have been undertaken in postpartum period and that too when some problems were detected. Research that has been conducted with dairy cows during the non-lactating period often has evaluated the effects of nutritional and managerial strategies for dry cows in preparation to improve the lactation performance. Only few data are available on the effects of herbal feed supplementation on the dry cow health, production, and reproduction during the periparturient period. Many of the strategies implemented during the dry period can affect the health and production performance of dairy cows in subsequent lactation.

Supplemention of chandrasoor (*Lepidium sativum* L.) and maithy (*Trigoella foenum graecum*) with the combination of jaggery and linseed oil once in every four days for a period of 60 days, improved reproductive performance of buffaloes by 85.71% and 71.42 %, respectively, in terms of estrus occurrence within the 60 days of experiment. The group supplemented with *chandrasoor*, exhibited prominently almost all symptoms of estrus. However, a feeble signs of estrus symptoms were seen in control group (Tomar, 1995 and Tomar *et al*., 1996).

Khajuria (1980) tried to enhance the reproductive performance by the supplementation of herbal preparation 'Banjh®' in cow and buffaloes. The animal came to heat 80% cases ($p<0.05$) on second day and 20% cases after second day course of treatment; pregnancy was found in 75% ($p<0.05$) of cases observed in heat. Similarly, Agrawal *and* Purbey (1981) treated anoestrus buffaloes with two 'Prajna' capsules daily for three consecutive days and observed 60% buffaloes in estrus in 23.4 days. The polyherbal formulation also improved the conception rate in anoestrus buffaloes (Shrivastava *et al*., 1983; Chaudhary and Purbey, 1983; Singh *et al.*, 2006).

Sharda (1998) administered 'Exapar®' at the rate of 100 ml per day for 2 to 4 days starting at parturition in cows and buffaloes and found that the expulsion of fetal membranes was significantly accelerated in treated group with no incidence of metritis as against 14.2% incidence in control. The average number of inseminations per conception (1.6 compared to 2.4 in control) and conception rate (100% compared to 80% in control) was better in the treated group. Singal, (1996); Dhakal (1999) and Arali (1999) observed that 'Exapar®' reduced time of onset of first post-partum estrus which might be due to significant early uterine involution in treatment group (32.61±2.33 days) in relation to control group (43.16±1.72 days).

2.6.3 Effect of shatavari on uterine health

Shatavari is reported to be the best female reproductive system tonic and toner (Brown, 1995). Supplementation of *shatavari* based polyherbal formulation in normal rats increased wet and dry uterine weights and also resulted in a marked increase in estrogen levels with no change in progesterone levels as compared to control. The primary changes in uterine tissues are controlled by estrogen and progesterone. The estrogenic effect in this case was observed only in the presence of functional ovaries

indicating that the shatavari per se does not possess any estrogenic activity. The effect is only evident in cases where the ovaries are functional, i.e., it improved the activity of ovary (Mitra *et al*., 1999) and regularized the estrous cycle (Nevrekar *et al*., 2002). This action can be attributed to the local healing of the endometrium (Nevrekar *et al*. 2002; Pandey *et al*., 2005).

Shatavari supplementation in dairy cows has been found to increase blood glucose (Berhane, 2000) and estrogen level (Mitra *et al*., 1999; Pandey *et al*., 2005) in rats. Improved level of blood glucose and estrogen level increase the flow of glucose in uterine endometrium; consequently there is more glycogen synthesis (Pandey *et al*., 2005). The energy source for the female reproductive system is estrogen dependent glycogen. Estrogen increases the glycogen content in the uterus and any decrease in uterine glycogen would directly implicate estrogen deficiency.

Shatavari has been found to cause an increase in uterine weight and uterine glycogen without altering serum estrogen and progesterone levels in immature rats as against ovariectomized rats used as control indicates that the phyto-estrogen performs its function by binding directly to the estrogen receptor without enhancing the endogenous estrogen levels (Gopumadhavan *et al*., 2005).

In dairy cows administration of estrogen is known to increase the wet weight of uterus significantly. Administration of *shatavari* based herbal formulation increased wet and dry uterine weight with marked increase in estrogen but not progesterone level as compared to control rats (Mitra *et al*., 1999). Elevated estrogen level stimulates and repairs uterine endometrium tissue and depots uterine glycogen. It has been reported that decrease in the estradiol 17-beta/ 17-alpha ratio resulted in a reduced rate of release of prostaglandins from the uterus and a slower rate of uterine involution (Erb *et al*., 1981). Besides, a higher concentration of biologically active estrogen may result in a faster rate of uterine involution due to increased PGF2 alpha release and *vice-versa* (Leslie, 1983).

Shatavari elevated estrogen level which stimulates and repairs uterine endometrium tissue and might be helpful to reduce the endometrial infection in dairy cows (Erb *et al*., 1981; Gopumadhavan *et al*., 2005; Pandey *et al*., 2005).

CHAPTER - 3

Materials and Methods

3.0 MATERIALS AND METHODS

The present study was conducted on Sahiwal heifers reared at livestock Research Centre, National Dairy Research Institute (NDRI) Karnal, Haryana from September 2011 to February 2012.

In the present study to investigate the effect of s*hatavari* supplementation on growth and sexual maturity sixteen Sahiwal heifers were selected from livestock research centre, NDRI Karnal. Experimental Sahiwal heifers were divided into two groups with eight animals in each on the basis of having similar range of body weight and similar age group (Table 3.2) and were kept in separate loose housing system. The experimental period was six months started from September (2011) to February (2012). The feeding schedule from September – December was Concentrate + Maize , Jowar while during January – February it was Concentrate +Oat ,Berseem for both groups with additional supplementation of *Shatavari* (@ 150 mg/ kg body weight / day) in treatment group (Table 3.4) In both groups concentrate and fodder were fed as per NRC (1989) requirements In the present study the effect of herbal feed supplement *Shatavari* on growth performance and sexual maturity of Sahiwal heifers was observed every 15 days interval during experimental period. The results obtained during the course of this study have been presented and discussed in respective.

3.1 LOCATION AND CLIMATIC CONDITION

Geographically NDRI livestock farm is situated at an altitude of 250 meters above the mean sea level in Indo-Gangetic alluvial plains on $29^0$42'N latitude and $72^0$02'E longitude. The climate of the farm is subtropical in nature. All groups of the animals were reared under similar climatic conditions.

3.2 Collection of *Shatavari* root powder

The herbs *Shatavari* (*Asparagus racemosus*) root powder 70 kg mesh was purchased from Raj & Company, Ehind Katju Market, Near Parsi Temple, Neemuch , Madhya Pradesh.(plate no.3.1). It used for feeding the experimental animals

3.3 SELECTION OF ANIMALS

The selected total sixteen Sahiwal heifers on the basis of having similar range of body weight and similar age group (table no.3.1).

Table 3.1 Body weight and similar age group of experimental animals

Groups	Body weight (kg)	Age (days)
Control	187.21±5.30	648.25±16.81
Treatment	192.32±7.27	656.25±16.81

3.4 Plan of work

The experiment was divided in to two groups G1 and G2

3.4.1 G1 (Control animals)

Traits considered for investigation

1. Dry matter of feed sample
2. DMI (kg)
3. Total Body weight change (fortnightly)
4. Body weight change (per day)
5. Observation of estrus in Sahiwal heifers

Plate 3.1a: Plant of *Shatavari* (*Asparagus racemosus*)

SHATAVARI PLANT

ROOTS

Plate 3.2b: Root powder and dry root of *Shatavari* (*Asparagus racemosus*)

DRY ROOTS

POWDER OF ROOTS

3.4.2 Group G1 (Treatment animals)

Traits considered for investigation

1. Dry matter of feed sample
2. Dry matter of offered Shatavari
3. DMI (kg)
4. Total Body weight change (fortnightly)
5. Body weight change (per day)
6. Observation of estrus in Sahiwal heifers

3.5 MANAGEMENT DURING EXPERIMENTAL TRIAL

3.5.1 Feeding management during upto sexual maturity

All animals were fed as per NRC (1989) during growing stage. During growing stage heifer were fed berseem and concentrate based minimum 1.5 kg concentrate per day per heifer (control diet). Depending upon the requirement of heifer, the quantity of concentrate was increased during the age of heifer. Shatavari was supplemented once in morning with the concentrate. The dose of shatavari was as given (Table no. 3.2).

Table 3.2 Feeding Schedule

	Group 1 (Control) (n= 8)	Group 2 (treatment) (n= 8)
September - December	Concentrate + Maize + Jowar	Concentrate + Maize + Jowar **+ Shatavari supplement @150mg / kg BW /animal/ day**
January - February	Concentrate +Oat +Berseem	Concentrate +Oat +Berseem **+Shatavari supplement @150mg / kg BW /animal/ day**

3.5.2 Housing management during Growing stage of Heifer

The control and experimental animals were maintained separately under loose housing and group management system. The paddocks, in which experimental animals were housed, were large, open and brick on edges flooring.

3.6 Estimation of body weight and body weight change

The fortnightly body weight of each animal was recorded early in the morning between 7:30 a.m. to 8:30 a.m. before providing the animals with any feeding stuff or water, using electronic weighing machine with a precision of 200g during the experimental period. To estimate the body weight change at different time interval, at the start of experiment, the animal's body weight were weighed before feeding and watering for two consecutive days to get their initial body weight. Thereafter, the weight of individual cow was recorded at fortnightly interval during the experimental period. Absolute rate of body weight change was estimated by using the following formula given by Broody (1945).

$$\textit{Absolute rate of change in body weight} = (W_2 - W_1)/(T_2 - T_1)$$

Where,

W_2 = Final

W_1 = Initial body weight

T_2-T_1 = the time interval in days

3.7. ESTIMATION OF FEED INTAKE

Fortnightly record of the amount of each feed given and left over was undertaken to get daily feed intake of each groups.

3.8 SAMPLE COLLECTIONS AND ANALYSIS

3.8.1 Feed and fodder

A measured quantity of sample was taken in pre-weighed moisture cup and then kept in hot air oven at 100 ±2 ^{oC} for 24 hours. Concentrate mixture, green fodder and dry feed was sampled fortnightly and analyzed for dry matter (DM).

The dry matter content was estimated as percentage of the sample taken.

$$\% DM = \frac{Weight\ of\ sample\ after\ dry}{Weight\ of\ sample\ before\ dry} \times 100$$

DMI = DM offered – DM residue

3.9 BLOOD SAMPLE COLLECTION AND PLASMA BIOCHEMICAL ASSAY

The blood samples were collected from jugular vein into heparinized (20 IU heparin/ ml blood) tubes from all experimental animals at fortnight interval form 0 day of start experiment to the end of experiment. Immediately after sampling the blood was placed in ice box and taken to the laboratory. To separate the plasma from the cells, blood samples were centrifuged at 3000 rpm for 15 to 20 minutes and stored frozen in deep freezer (-20°C) until analyzed.

3.9.1 Estimation of growth hormone

This Enzyme immunoassay (EIA) is based on the competition between unlebelled rat growth hormone (rGH) and acetylcholinesterase (AChE) linked to rat growth hormone (tracer) for limited specific goat anti-rat GH antiserum sites.

The complex goat antiserum –rat GH (free GH or tracer) bind to the rabbit polyclonal antibody anti goat immunoglobin antibodies that are attached to the well.

The plate is then washed and Ellman's Reagent (Enzymatic substrate for AChE and chromogen) is added to the well .

The AChE tracer act on the Ellman's Reagent to form a yellow compound.

The intensity of the color , which is determined by spectrophotometry , is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free rat GH present in the well during the immunological incubation.

3.9.1.1 REAGENT PREPARATION

1. EIA buffer

   Reconstitute one vial with 50 ml of dist or deionized water .Use a magnetic stirrer to mix the contents. Stability at 4 °C: 1 month.

2. Rat GH standard (calibrated against the reference preparation NIDDK standard Rgh-RP2)

Reconstitute the vial with 1 ml of distilled or deionized water .Allow it to stand 5 minute until completely dissolved and mix thoroughly by gentle inversion . The concentration of the first standard is 40 ng/ml . Prepare seven propylene tube (for the seven other standards) and add 500 µLof EIA buffer into each tube. Add 500 µLof the first tube (contain the first standard) to the second tube. Continue this procedure for the other tubes .thus, standard concentrations are:40(S1), 20(S2) ,10(S3) ,5(S4) ,2.5(S5), 1.25(S6), 0.63(S7) and 0.31 ng/ml (S8), respectively. Stability at 4 °C:1 week.

3. Quality control (on day 1)

   Reconstitute one vial with 1ml of distilled or deionized water .allow it to stand 5 minute unit completely dissolved and then mix thoroughly by gentle inversion .stability at 4 °C:1 week.

4. Rat GH antiserum (on day 1)

   Reconstitute one vial with 5ml of EIA buffer .allow it to stand 5 minute unit completely dissolved and then mix thoroughly by gentle inversion . stability at 4 °C:1 week.

5. Wash buffer (on day 1)

   Dilute 1ml of the concentrated Wash buffer to 400 ml with distilled or deionized water. Add 200 µL of tween 20 (use a magnetic stirrer to mix the contents). Stability at 4 °C:1 week.

6. Rat GH-AChE tracer (on day 2)

   Reconstitute one vial with 5ml of EIA buffer .allow it to stand 5 minute unit completely dissolved and then mix thoroughly by gentle inversion. Stability at 4 °C: 1 month.

7. Ellman’s reagent (on day 3)

   Five minute before use, Reconstitute with 50 ml of distilled or deionized water. The tube contents should be thoroughly mixed. Stability at 4 °C and in dark: 4 days.

3.3 ENZYME IMMUNOASSAY PROTOCOL OF GROWTH HORMONE

ENZYME IMMUNOASSAY PROTOCOL (volume are in µL)					
	Blank	**Non Specific Binding**	**Maximum Binding**	**Standard**	**Sample**
Buffer	**-**	**100**	**50**	**-**	**-**
Standard	**-**	**-**	**-**	**50**	**-**
Sample	**-**	**-**	**-**	**50**	**50**
Antiserum	**-**	**-**	**50**	**-**	**50**
	Cover the plate, incubate at 20 °C for 20 h				
Tracer	**-**	**50**	**50**	**50**	**50**
	Cover the plate, incubate at 20 °C for 20 h				
	Wash the plate 5 times				
Ellman's reagent	**200**	**200**	**200**	**200**	**200**
	Incubate the plate with an orbit shaker in the dark at room temperature				
Read the plate between 405 and 414 nm					

3.9.1.2 STATISTICAL ANALYSIS OF DATA

- Data were subjected to analysis by ANOVA of two way classification
- Data were expressed as Mean ± SE
- Descriptive Statistical
- For comparison of mean- t-test

3.9.2 Estimation of cortisol hormone

3.9.2.1 Introduction

Cortisol is glucocorticoid produced by the adrenal cortex in response to adrenocorticotropic hormones (ACTH). Cortisol is secreted with a cicardian periodicity and peaks just prior to waking in morning. The production of glucocorticoids is increased by stress; therefore cortisol can be used as a biomarker of stress. Cortisol levels increase with age and are often elevated in major depressive disorder , certain forms of hypertension and AIDS. Pharmacological treatment with glucocorticoids can results in cognitive impairment, decreased bone density, hypertension, and an increased risk of development of type II diabetes.

Cortisol binds to two intracellular receptor , the mineralocorticoid receptor (MR) , and the glucocorticoids receptor (GR). Of the two receptor ,the MR has the higher affinity for cortisol. This receptor will be almost completely occupied by cortisol at levels too low to activate the GR. 11 B- hydroxysteroid dehydrogenase (type 2) (11b-HSD2) converts cortisol to inactive cortisone .this enzyme is expressed predominantly in mineralocorticoid target tissue including kidney, colon and salivary gland where it serves to protect the MR from glucocorticoids excess. Individual lacking this enzyme exhibit a syndrome known as apparent mineralocorticoid excess which features hypertension and hypokalemia.

The enzyme 11b-HSD1 is a key regulator of intracellular glucocorticoids level , catalyzing the regeneration of cortisol from cortisone. Visceral adipose tissue from obese humans has increased 11b-HSD1 activity compared to adipose tissue obtained from normal individuals. Cortisol strongly promotes adipocyte differentiation; mature visceral adipocytes express high levels of the glucocorticoids receptor.

Cortisol can be measured in many matrices including blood, feces, urine, and saliva. Serum cortisol concentration range from about 25-800 nM(9-300 ng/ml) and approximately 90-95% of the cortisol is bound to proteins . urinary cortisol is not bound to proteins, but its levels are dependent on glomerular and rubular function .in saliva, approximately 67% of cortisol is unbound. There is generally good correlation between cortisol measurements in saliva and serum.

3.9.2.2 Preparation of Assay- specific reagents

3.9.2.3 Cortisol EIA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip ,transfer 100 µL of the cortisol EIA Standard (Catalog No. 400364) into a clean test tube , then dilute with 900 µL ultra pure water. The concentration of this solution (the bulk standard) will be 40 ng/ml. It will be stable for at least six weeks.

To prepare the standard for use in EIA : obtain eight clean test tubes and numbers them #1 through #8 . Aliquot 900 µL EIA buffer to tube #1 and 600 µL EIA buffer to tubes #2-8. Transfer 100 µL of the bulk standard (40ng/ml) to tube #1 and mix thoroughly. The concentration of this standard , the first point on the standard curve, will be 4 ng / ml (4,000pg/ml).serially dilute the standard by removing 400 µL from tube #1 and placing in tube #2; mix thoroughly ,next, remove 400 µL from tube #2 and place it into tube #3 ; mix thoroughly . repeat this process for tubes #4-8. This dilute standard should not be stored for more than 24 hours.

3.9.2.4 Cortisol AChE Tracer

Reconstitute the cortisol AChE Tracer as follows:

100 dtn Cortisol AChE Tracer (96- well kit; catalog No.10005272): reconstitute with 6 ml EIA Buffer. Store the reconstituted cortisol AChE Tracer at 4 °C (do not freeze) and use within four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

3.9.2.5 Cortisol EIA Monoclonal Antibody

Reconstitute the cortisol EIA Monoclonal Antibody as follows:

100 dtn Cortisol EIA Monoclonal Antibody (96- well kit; catalog No. 400362):

Reconstitute with 6 ml EIA Buffer

Store the reconstituted cortisol EIA Monoclonal Antibody at 4 °C.It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

3.9.2.6 STATISTICAL ANALYSIS OF DATA

- **Data were subjected to analysis by ANOVA of two way classification**
- **Data were expressed as Mean ± SE**
- **Descriptive Statistical**
- **For comparison of mean- t-test**

Table no. 3.4 ENZYME IMMUNOASSAY PROTOCOL OF CORTISOL

Steps	Reagent	Blank	TA	NSB	B_0	Std/ Sample
Add Reagents	EIA Buffer	-	-	100 µL	50 µL	-
	Standard/Sample	-	-	-	-	50 µL
	Tracer	-	-	50 µL	50 µL	50 µL
	Antibody	-	-	-	50 µL	50 µL
Incubate	Cover plate and incubate overnight at 4 °C.					
Wash	Wash all wells five times					
Add reagents	Tracer	-	5 µL	-	-	-
	Ellman's	200 µL	200 µL	200 µL	200 µL	200 µL
Incubate	Cover plate and incubate 90- 120 minute RT with gentle shaking					
Read	Read plate at a wavelength between 405 -420 nm					

3.9.3. Estimation of Progesterone

3.9.3.1 Introduction

Progesterone is a steroid hormone (C21 steroid, pregn-4-ene-3, 20 dione) and is synthesized from tissue and circulating cholesterol. The principal production sites are the adrenals and ovaries and placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by kidneys.

The primary role is played in reproductive organ. In males the Progesterone plays an intermediary role in the synthesis of corticosteroids and androgens. In females, progesterone remains relatively constant through follicular phase of ovulation. Then the levels increase following ovulation and remains elevated for 4-6 day decreasing to the base line 24 hours before the onset of cycle. In pregnancy, placental progesterone raise 10-30 times those of the luteal peak levels. In females, the measurement of progesterone is useful in evaluating the status of ovarian factions. Monitoring of progesterone therapy and early stage pregnancy evaluations comprise the remainder of progesterone assays. During early ovarian maturation progesterone levels increase progressively in girls, in parallel with increase in gonadotropins. The importance of sequential progesterone measurement for monitoring ovulation induction, particularly in “in vitro” fertilization programs has recently been reported. The monitoring of LH and progesterone will help the breeders.

3.9.3.2 Test principles

The progesterone quantitative test is based on a solid- phase enzyme immunoassay based on competitive binding method. A sample (serum/ plasma) containing an unknown amount of progesterone will complete with enzyme-conjugated progesterone for high affinity binding sites on a limited numbers of antibodies coated on to the plate. After washing away the free antigen, the amount of labeled antigen in the sample is reversibly proportional to the concentration of the unlabeled antigen. The actual concentrations is unknown samples are obtained by means of a standard curve based on known concentrations of unlabeled antigen analyzed in parallel with the unknowns. After5 washing, substrate solution is added and the enzyme allowed to react for a fixed time before the reaction is terminated. Absorbencies are measured at 450 nm using ELISA plate reader. A standard curve

is produced using values from standards which absorbency values for blank tubes have been subtracted. Results for unknown may be read directly from this standard curve using either manual calculation or by a suitable computer program. This kit suitable for the direct measurement of progesterone in serum / plasma samples.

3.9.3.3 Reagent preparation

1. Prepare Wash buffer by diluting 1part with 19 parts of distilled water, excess amount may be stored at 2-8 ^{o}c for couple of weeks.

2 Dilute highly concentrated specimen samples with dilution buffer and mix well before use in the assay.

3. Standard solution, if not used immediately, should be kept frozen at

3.9.3.4 STATISTICAL ANALYSIS OF DATA

- Data were subjected to analysis by ANOVA of two way classification
- Data were expressed as Mean ± SE
- Descriptive Statistical
- For comparison of mean- t-test

CHAPTER – 4

Results and Discussion

4.0 RESULTS AND DISCUSSION

Shatavari (*Asparagus racemosus)* is the most commonly used traditional medicine in human beings and its supplementation are recommended as appetizer, reproductive and rejuvenative tonic and, also for hormonal balances, weight gain in women. It is also use as polyherbal supplementation fed for kids that increases growth rate and weaning weight. Keeping in view these benefits, *shatavari* herb was selected as feed supplement to investigate its effect on growth and sexual maturity of Sahiwal heifers.

In the present study to investigate the effect of shatavari supplementation on growth and sexual maturity sixteen Sahiwal heifers were selected from livestock research centre, NDRI Karnal. Experimental Sahiwal heifers were divided into two groups with 8 animals in each on the basis of having similar range of body weight and similar age group and were kept in separate loose housing system. The experimental period was six months started from September (2011) to February (2012). The feeding schedule from September – December was Concentrate + Maize + Jowar while during January – February it was Concentrate +Oat +Berseem for both groups with additional supplementation of *Shatavari* (@ 150 mg/ kg body weight /animal/ day) in treatment group (Table 3.2) In both groups concentrate and fodder were fed as per NRC (1989) requirements (proportions on DM basis). In the present study the effect of herbal feed supplement Shatavari on growth performance and sexual maturity of Sahiwal heifers was observed every 15 days interval during experimental period. The results obtained during the course of this study have been presented and discussed in respective phases.

4.1 Effect of s*hatavari* supplementation on dry matter intake

The proximate composition of *shatavari* fed to treatment Sahiwal heifers during experimental period is presented in Table 4.1. The overall average values of DMI (kg/day) during six months of experimental period were 4.70±0.09 and 5.35±0.18 in control and treatment group, respectively (Table 4.1). The overall average value of DMI (kg/day) of treatment group was significant at 5% level of significance. There was significant increase in dry matter intake (kg/day) in treatment group compare to control groups from fifth fortnight onward upto thirteenth fortnight. The DMI was 4.48 ±0.20 vs. 5.18 ±0.21kg/day, 4.61 ±0.21 vs. 5.18±0.21kg/day, 4.84 ±0.20 vs. 5.32±0.22 kg/day, 4.42 ±0.21 vs. 5.23±0.15 kg/day, 4.77±0.19 vs. 5.65±0.18kg/day, 5.97 ±0.22 vs. 5.81±0.22 kg/day, 5.08 ±0.24 vs. 5.97±0.19 kg/day, 5.23 ±0.21 vs. 6.27±0.19 kg/day, 5.53±0.22 vs. 6.33±0.18 kg/day respectively. The DMI was 4.48^{a} ±0.20 vs. 5.18^{b}±0.21 kg/day, the significant increase in overall dry matter intake (kg/day) in treatment group was 2.40 kg per 100 kg body weight which is under the standard value of 2.5 kg /100 kg body weight. Shatavari is a well documented digestive tonic, appetizer, effective in dyspepsia and indigestion (Dalvi *et al*., 1990, Kumar *et al.,* 2006). As reported by Mahantra *et al.*, 2003 feeding a herbal formulation containing 25% *shatavari* enhanced dry matter intake significantly by 10.97% in buffaloes. There are many repoters stating *Shatavari* caused increase in DMI might be due to improved digestibility of nutrients (*In Vitro)* (Bakshi *et al.,* 2004; Wadhwa and Bakshi, 2006; Kumar *et al.*, 2006). Thus observed increase dry matter intake in treatment groups in present study due to *shatavari* supplementation is in accordance with early reports.

Table 4.1 Average dry matter intake (DMI) (kg/day) (P<0.05)

Fortnight	Group			
	Control		Treatment	
	(Mean ± SE)	(%)	(Mean ± SE)	(%)
1	4.28±0.16	2.29	4.41±0.19	2.27
2	4.29±0.17	2.26	4.44±0.18	2.25
3	4.31±0.16	2.23	4.65±0.17	2.31
4	4.51±0.33	2.30	5.01±0.18	2.45
5	4.48^{a} ±0.20	2.24	5.18^{b}±0.21	2.42
6	4.61^{a} ±0.21	2.27	5.34^{b}±0.18	2.47
7	4.84^{a} ±0.20	2.28	5.32^{b}±0.22	2.40
8	4.42^{a} ±0.21	2.11	5.23^{b}±0.15	2.32
9	4.77^{a} ±0.19	2.24	5.65^{b}±0.18	2.41
10	5.97^{a} ±0.22	2.30	5.81^{b}±0.22	2.44
11	5.08^{a} ±0.24	2.31	5.97^{b}±0.19	2.45
12	5.23^{a} ±0.21	2.34	6.27^{b}±0.19	2.51
13	5.53^{a} ±0.22	2.36	6.33^{b}±0.18	2.47
Overall	**4.70^{a} ±0.09**	**2.27**	**5.35^{b}±0.18**	**2.40**

Mean with different superscripts within a row differ significantly (P<0.05)

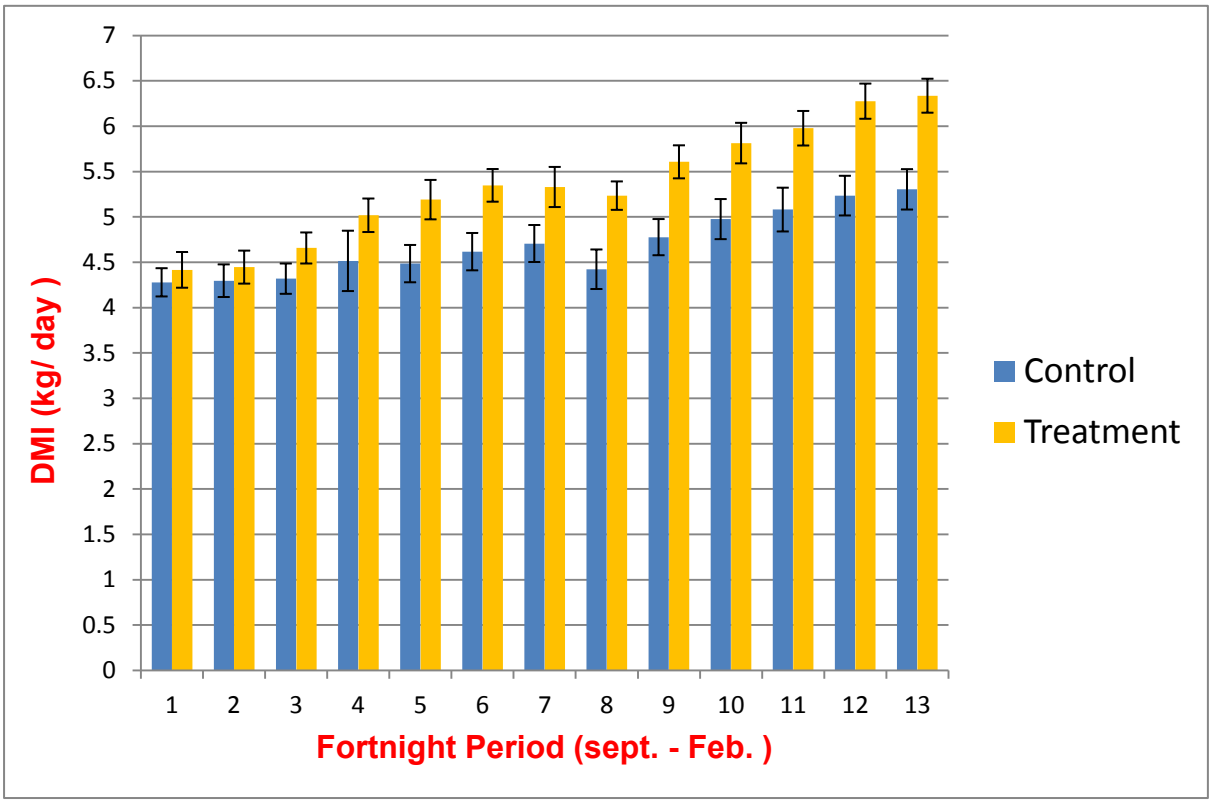

Fig. 1 Average dry matter intake (kg/day) of both groups

4.2 Effect of shatavari supplementation on growth performance

4.2.1 Body weight

The overall average values of body weight (kg) during six months of experimental period was 206.74 ± 3.46 and 222.68 ± 5.7 in control and treatment group, respectively (Table 4.2). The increase in overall average values of body weight (kg) of treatment group was significant at 5% level of significance. The body weight of control and treatment group was significant at 90,105,120,135,150,165 and 180 day was 206.61 ±5.38 vs. 221.60 ±6.03 (kg), 210.52 ±5.60 vs. 226.40±6.23 (kg) , 213.73 ±5.17 vs. 230.73 ±6.40 (kg) , 216.73 ±5.86 vs. 235.90 ±6.27 (kg) , 219.9 ±6.12 vs. 241.51 ±6.25 (kg), 223.25 ±6.26 vs. 248.16±6.23 (kg), 226.40 ±6.47 vs. 256.23±6.15 (kg) respectively. There was significant increase in body weight (kg) in treatment group compare to control groups from seventh fortnight onward upto thirteenth fortnight.

This significant increase in body weight (kg) in treatment group in the present study was in agreement with Mirzaei and Prasad (2011) and Sharma (2011). Mirzaei and Prasad (2011) in their study of polyherbal supplementation feeding which included *shatavari* also on goats reported that due to polyherbal supplementation kid weaning weight was improved compare to non polyherbal supplementation. Sharma (2011) reported significant increase of weight of ovaries, uterus, breast and teats in female rats due to feeding supplementation of *Shatavari* @30 mg/100g bw. The reported that feeding of sarsaponin cuased increase body weight of growing pigs and steers (Mader *et al.,*1987). Gupta *et al*. (2004) supplemented shatavari with the combination of *Cryptolepis buchanani* at the rate of 1% of DM as feed additive and reported significant improvement in digestibility of feed nutrients consequently significant higher body weight gain in supplemented female calves as compared to un-supplemented group. Thus observed increase of body weight (kg) in treatment groups of Sahiwal heifers in present study may be due to *Shatavari* supplementation.

Table 4.2 Average body weight (kg) of Sahiwal heifers in fortnight (September to February) (P<0.05)

Fortnight	Group	
	Control (Mean ± SE)	**Treatment (Mean ± SE)**
1	187.21±5.30	192.32±7.27
2	190.76±4.39	197.17±6.84
3	193.90±5.08	201.16±6.61
4	196.51±5.72	204.85±6.33
5	200.20±5.25	210.28 ±5.98
6	203.5±5.54	216.18 ±5.85
7	206.61^{a} ±5.38	221.60^{b} ±6.03
8	210.52^{a} ±5.60	226.40^{b} ±6.23
9	213.73^{a} ±5.17	230.73^{b} ±6.40
10	216.73^{a} ±5.86	235.90^{b} ±6.27
11	219.9^{a} ±6.12	241.51^{b} ±6.25
12	223.25^{a} ±6.26	248.16^{b}±6.23
13	226.40^{a} ±6.47	256.23^{b}±6.15
Overall	**206.74^{a} ± 3.46**	**222.68^{b} ± 5.7**

Mean with different superscripts within a row differ significantly (P<0.05)

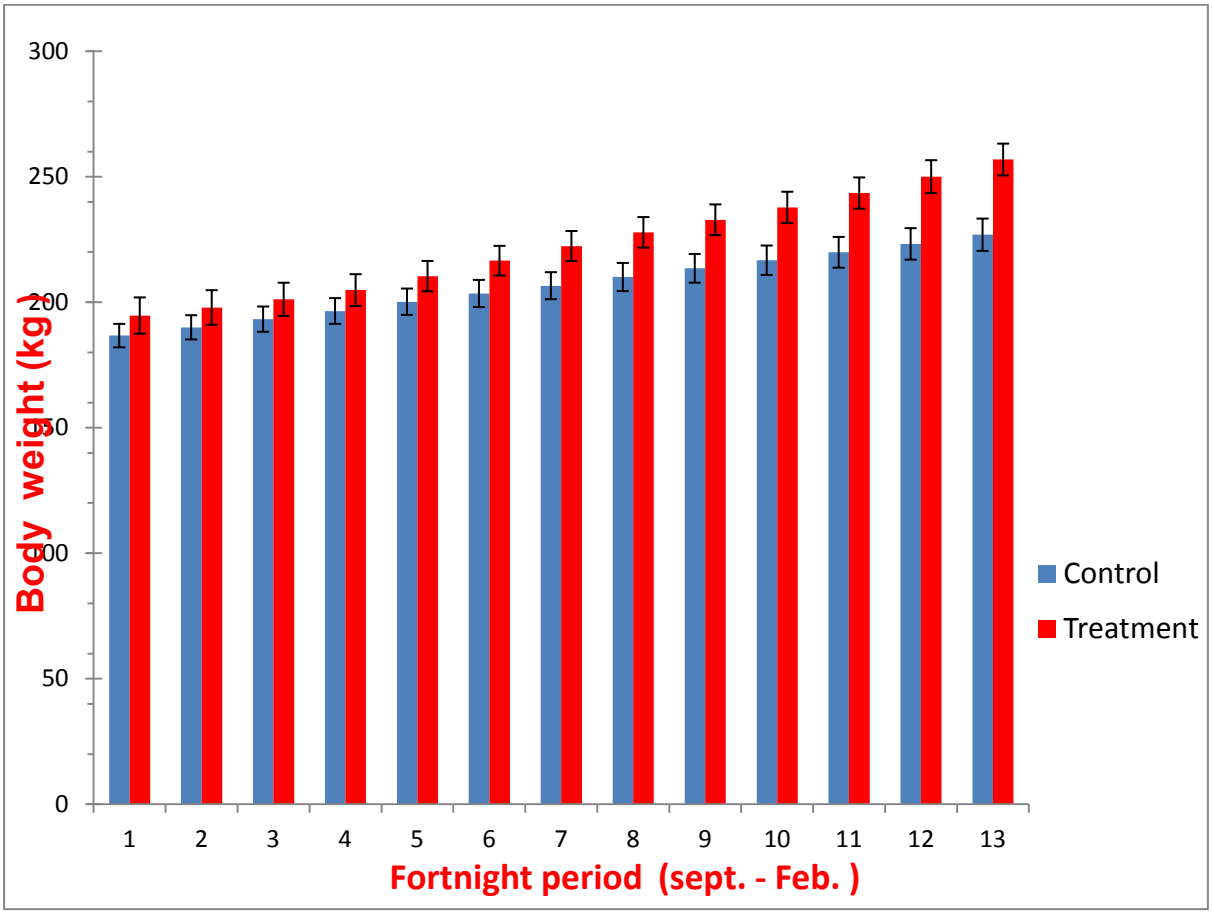

Fig. 2 Average body weight (kg) of Sahiwal heifers in fortnight (September to February)

4.2.2 Average Daily Gain (gm/day)

The overall average values of average daily gain (ADG) during six months of experimental period was 223.26 ± 21.53 and 345.34 ± 23.40 in control and treatment group, respectively (Table 4.3). During the experiment period highest growth rate in treatment group was observed in months of February (11^{th} and 12^{th} fortnight)(fig. no.4). The overall average value of growth rate (gm/day) of treatment group was significant at 5% level of significance. There was significant increase in growth rate (gm/day) in treatment group compare to control groups from 5^{th} fortnight onward upto 12^{th} fortnight from 60,75,90,105,120,135,150,165, and 180 days. The growth rate was 220 ±34.75 vs.410±46.08 , 206.66 ±30.44 vs. 389.16±49.56 , 213 ±26.69 vs. 363.33±29.00, 231.66 ±20.46 vs. 334.16±19.85, 214.16 ±18.27 vs. 330.83±20.42 , 210.83 ±28.33 vs. 379.16±38.77 , 223.33±23.46 vs. 435.02±38.76 , 243.33±29.78 vs. 455.05±35.42 gm/day respectively(fig. no. 3). This increase in growth rate was also confirmed by the observed higher growth hormone (GH) level in treatment group (Table 4.4) compared to control group during the same period.

These finding in the present study are in accordance with Mirzaei and Prasad (2011). Mirzaei and Prasad (2011). In their study of polyherbal supplementation feeding, which included shatavari, on goats reported that due to polyherbal supplementation kid growth rate was improved compare to non polyherbal supplementation. Thus observed growth rate in treatment groups of Sahiwal heifers in the present study could be attributed to *Shatavari* supplementation.

Table 4.3 Average daily gain (gm/day) of Sahiwal heifers (P<0.05)

Fortnight	Group	
	Control (Mean ± SE)	**Treatment (Mean ± SE)**
1	220±14.25	215±25.34
2	217.5±23.39	215.83±23.56
3	215.83±28.33	245.83±41.62
4	245.83±26.91	370.83±55.21
5	220^{a} ±34.75	410^{b}±46.08
6	206.66^{a} ±30.44	389.16^{b}±49.56
7	213^{a} ±26.69	363.33^{b}±29.00
8	231.66^{a} ±20.46	334.16^{b}±19.85
9	214.16^{a} ±18.27	330.83^{b}±20.42
10	210.83^{a} ±28.33	$379.16^{b.}$±38.77
11	223.33^{a} ±23.46	435.02^{b}±38.76
12	243.33^{a} ±29.78	455.05^{b}±35.42
Overall	**223.26^{a} ±21.53**	**345.34^{b}±23.40**

Mean with different superscripts within a row differ significantly (P<0.05)

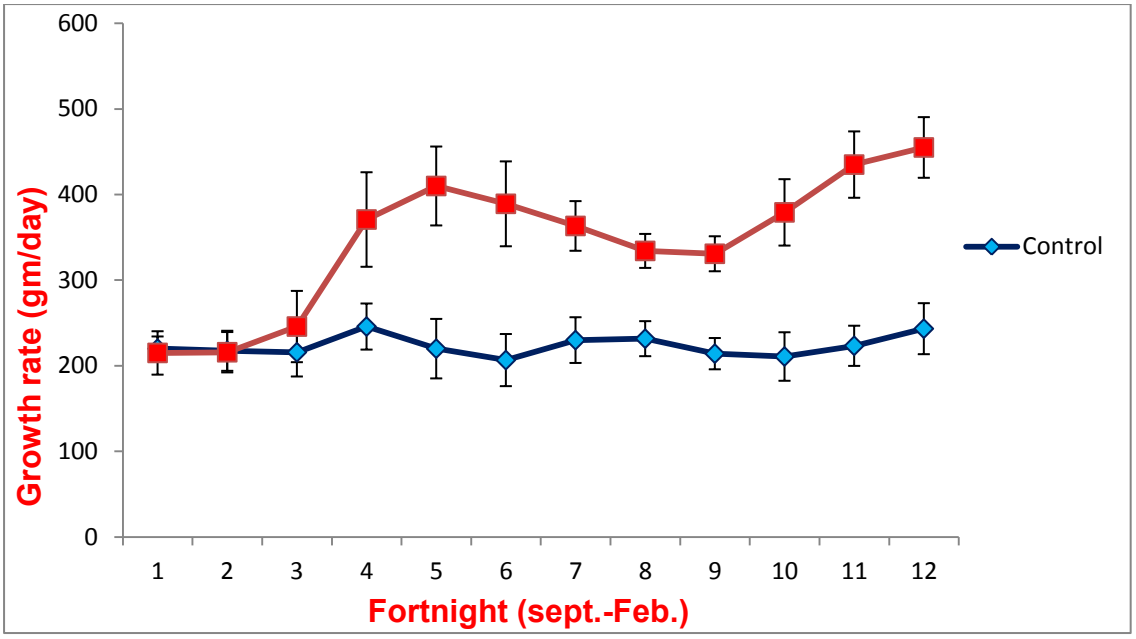

Fig. 3 Average daily gain (gm/day) of Sahiwal heifers (P<0.05)

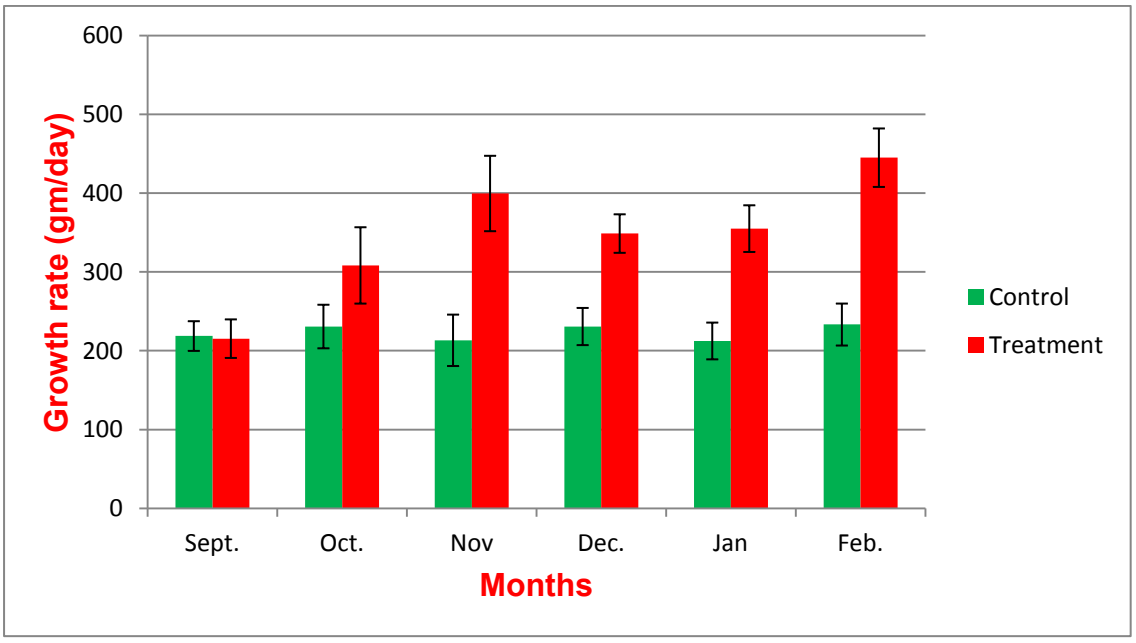

Fig. 4 Average daily gain (gm/day) in month of September to February Sahiwal heifers (P<0.05)

4.3 Effect of shatavari supplementation on growth hormone

The overall average level of growth hormone (ng/ml) during six months of experimental period was 5.41±0.15 and 6.33±0.11 in control and treatment group, respectively (Table 4.4). The overall average level of growth hormone (ng/ml) of treatment group was significant at 5% level of significance. There was significant high level of growth hormone (ng/ml) in treatment group compare to control groups from 7^{th} fortnight onward upto 12^{th} fortnight except at 9^{th} fortnight on day 90, 105,135,150,165 and 180 day. The concentration of GH was 5.17±0.17 vs. 7.10±0.61ng/ml, 5.23±0.58 vs. 6.819±0.00 ng/ml, 4.59±0.24 vs. 6.22±0.36ng/ml, 5.02±0.50 vs. 6.10±0.0.30ng/ml, 5.10±0.39 vs. 6.53±0.39ng/ml, 5.37±0.36 vs. 6.37±0.33 ng/ml respectively. This rise in GH (ng/ml) level in treatment group can also be observed by higher growth rate (gm/day) in this group. This observed improvement in both growth rate and GH level in treatment groups could be credited to *Shatavari* supplementation.

4.4; Effect of shatavari supplementation on cortisol (ng/ml)

The overall average level of cortisol (ng/ml) during six months of experimental period was 3.52±0.068 and 3.44±0.08 in control and treatment group, respectively (Table 4.5). Thus in the present study there was no significant change observed in both control and treatment group in cortisol level. Cortisol is considered to be indicator of biological stress (Cole and Cupp 1977,Rao and Pandey 1981,Iyimo *et al.,*2007). *Shatavari* has anti-stress properties (Kumar *et al.,* 2008) this indicates that Shatavari supplementation don't cause any stress in cattle.

Table 4.4 Growth hormone (ng/ml) (P<0.05)

Fortnight	Group	
	Control (Mean ± SE)	**Treatment (Mean ± SE)**
1	6.10±0.38	6.20±0.32
2	6.34±0.57	6.04±0.48
3	6.26±0.56	6.15±0.48
4	5.84±0.37	6.00±0.37
5	5.45±0.21	5.97±0.217
6	5.49±0.19	6.63±0.43
7	5.17±^{a}0.17	7.10^{b}±0.61
8	5.23^{a}±0.58	6.819^{b}±0.00
9	4.81±0.29	5.85±0.35
10	4.59^{a}±0.24	6.22^{b}±0.36
11	5.02^{a}±0.50	6.10^{b}±0.0.30
12	5.10^{a}±0.39	6.53^{b}±0.39
13	5.37^{a}±0.36	6.37^{b}±0.33
Overall	**5.41^{a}±0.15**	**6.33^{b}±0.11**

Mean with different superscripts within a row differ significantly (P<0.05)

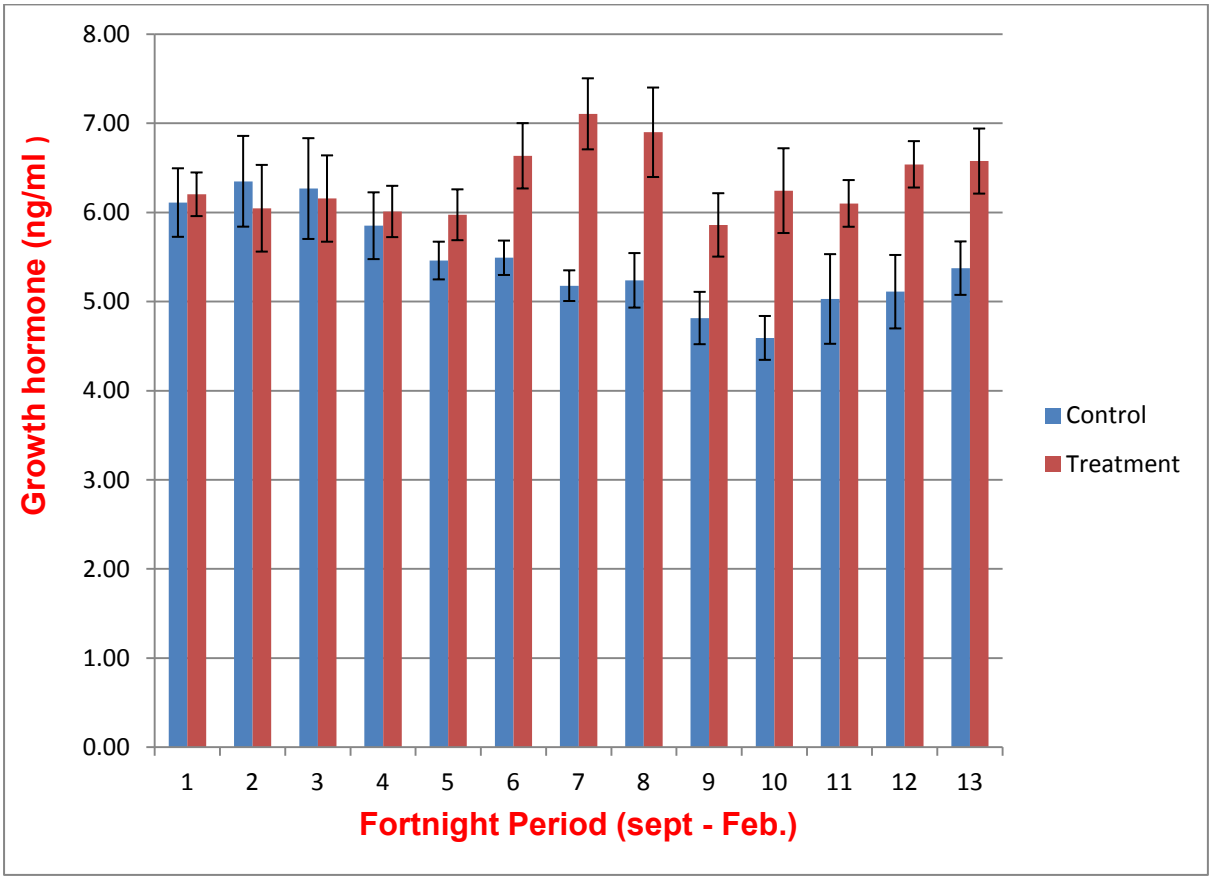

Fig. 5 Growth hormone (ng/ml)

Table 4.5 Cortisol (ng/ml) (P<0.05)

Fortnight	Group	
	Control (Mean ± SE)	**Treatment (Mean ± SE)**
1	3.95±0.32	3.70±0.12
2	3.64±0.18	3.67±0.10
3	3.40±0.24	3.54±0.24
4	3.37±0.41	3.49±0.29
5	3.54±0.39	3.10±0.19
6	3.10±0.39	3.26±0.22
7	3.20±0.41	3.37±0.35
8	3.89±0.29	3.86±0.35
9	3.78±0.30	3.42±0.13
10	3.45±0.54	3.64±0.34
11	3.90±0.24	3.51±0.23
12	3.15±0.65	3.24±0.23
13	3.43±0.16	3.01±0.26
Overall	**3.52±0.068**	**3.44±0.08**

Mean with different superscripts within a row differ significantly (P<0.05)

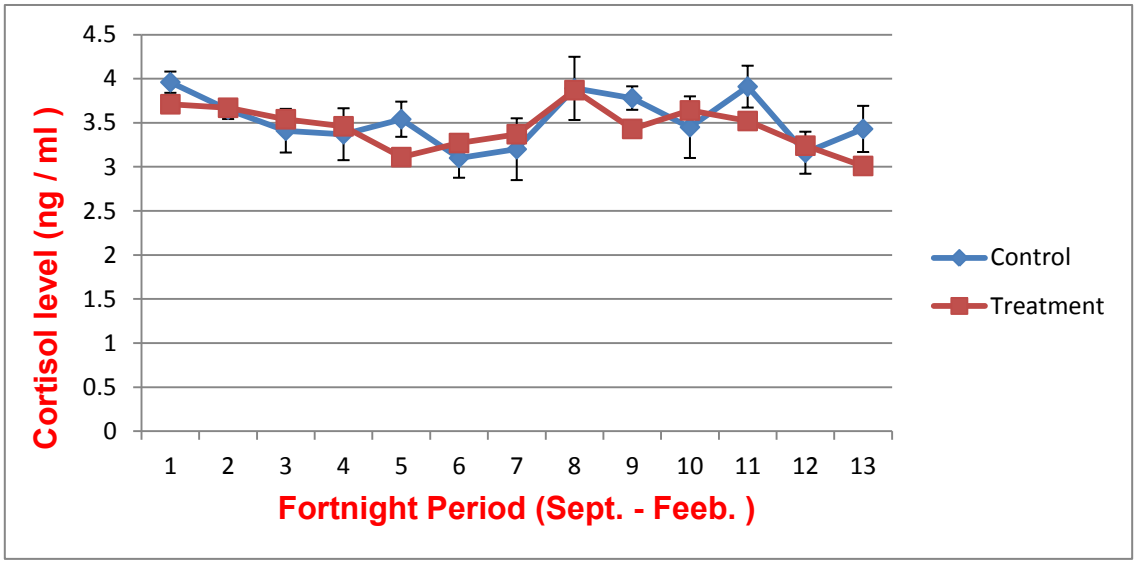

Fig. 6 Cortisol (ng/ml) concentration in Sahiwal heifers

4. 5 Effect of *Shatavari* supplementation on age at puberty of Sahiwal heifers

To observe the effect of *Shatavari* supplementation on age at puberty of Sahiwal heifers the progesterone level (ng/ml) was recorded for all thirteen fortnight in both control and treatment group as shown in figure no. 7 and 8 respectively and, also the date of first heat was recorded for individual animal in both control and treatment group. The average age at puberty of Sahiwal heifers in control and treatment group was 739.66±19.17 and 713.60±16.10, respectively (Table 4.6). The average age at puberty of Sahiwal heifers of treatment group was significant at 5% level of significance. Also the progesterone level was (>0.5 ng/ml) (table no.4.7 & 4.8) in both groups on date of first heat. During experimental duration two animals from treatment and one animal from control was excluded from the determination of age at puberty due to their value > 0.5 ng/ml. In the study of Berhane(2000), reported that supplementation of Shatavari @100g on alternate day and observed that 100 % in estrus and 75 % conception in treatment group compared to control 50% in estrus within 90 days of parturition.

Table 4.6 Age at puberty of Sahiwal heifers

Group	Age at puberty (Days)
Control	739.66^{a} ±19.17
Treatment	713.60^{b} ±16.10
***P* value**	0.05

Table No. 4.7 The Progesterone level (ng/ml) in treatment groups of Sahiwal heifers

Animal no.	1 Dec.2011	16 dec.2011	1 Jan. 2012	16 Jan. 2012
1	1.45	0.85	1.43	1.58
2	0.23	0.50	1.08	0.70
3	0.40	1.25	0.89	1.69
4	0.28	1.34	0.84	1.37
5	0.70	0.50	1.30	1.16
6	0.08	0.20	0.26	0.88
7	0.30	0.71	0.43	1.50
8	0.40	0.18	0.30	0.26

Table No. 4.8 The Progesterone level (ng/ml) in control groups of Sahiwal heifers

Animal no.	1 Dec.2011	16 dec.2011	1 Jan. 2012	16 Jan. 2012
1	1.17	1.01	1.56	1.30
2	0.30	0.82	0.58	0.60
3	0.30	0.41	1.05	1.05
4	0.20	0.21	0.21	0.95
5	0.03	0.38	1.15	0.60
6	0.08	0.23	0.20	0.28
7	0.31	0.33	1.04	1.35
8	0.08	0.23	0.22	0.42

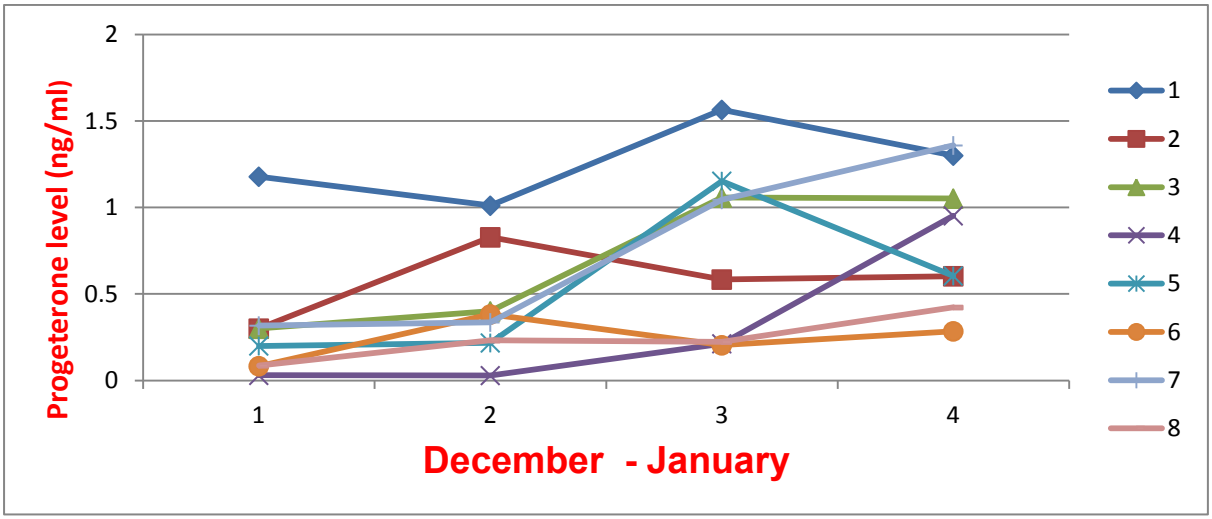

Fig. 7 Progesterone (ng/ml) level in control group

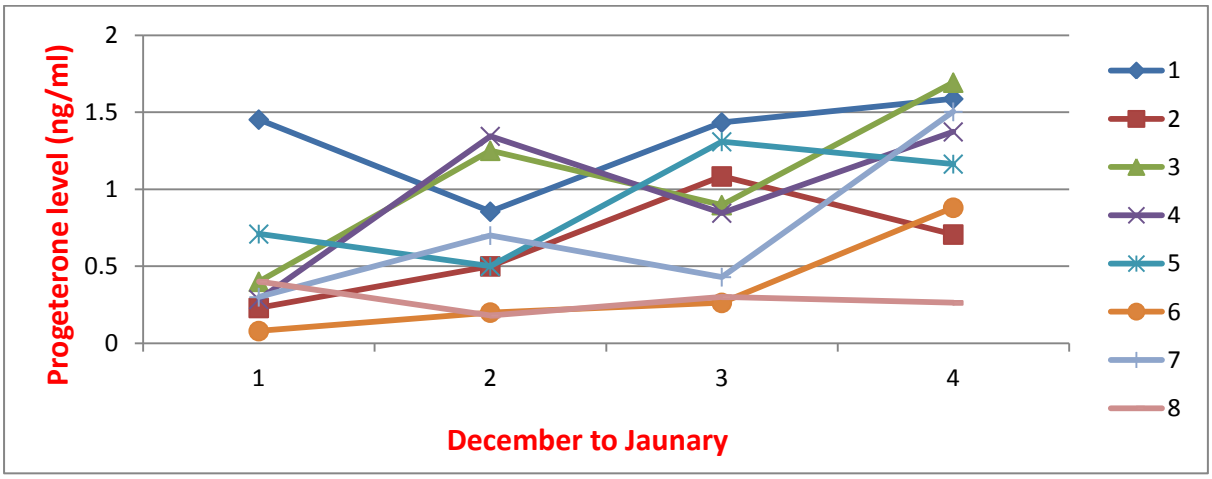

Fig. 8 Progesterone (ng/ml) level in treatment group

4.6 Effect of *Shatavari* supplementation on age at first service of Sahiwal heifers

To observe the effect of *Shatavari* supplementation on age at first service in Sahiwal heifers the date of first service was recorded in both control and treatment group. It was found that 37.5 % and 75 % Sahiwal heifers were at the age at first service in control and treatment group, respectively during experimental period (Table 4.9). The average age at first service of Sahiwal heifers in control and treatment group was 846.10±24.0 and 817.40±20.37, respectively (Table 4.10). The average age at first service of Sahiwal heifers of treatment group was significant at 5% level of significance. Higher % of animal age at first service (75 %) and lower average age at first service (813.40±20.37). The polyherbal formulation also improved the conception rate in anoestrus buffaloes (Shrivastava *et al*., 1983; Chaudhary and Purbey, 1983; Singh *et al.*, 2006). Berhane, (2000) reported that supplementation of *Shatavari* (100g on alternate day) postpartum alone led to 100% estrus and 75% conception in treatment group as compared to 50% in control crossbred cow within 90 days of calving.

Table 4.9 Percentage of animals in first service

Group	% of animal age at first service
Control (n=8)	37.5
Treatment (n=8)	75

Table 4.10 Age at first service in days

Group	Age at first service (Days)
Control	$846.10^{a} \pm 24.0$
Treatment	$817.40^{b} \pm 20.37$
P value	0.05

CHAPTER – 5

Summary and Conclusions

5.0 SUMMARY AND CONCLUSION

In India's Vedic scripture *Shatavari* (*Asparagus racemosus)* has been considered the best herbs in *Ayurveda* and this amazing herb is known as the "queen of herbs". *Shatavari* is the most commonly used traditional medicine in human beings and its supplementation are recommended as appetizer, reproductive and rejuvenative tonic and, also for hormonal balances, weight gain in women. It is also use as polyherbal supplementation fed for kids that increases growth rate and weaning weight but no study has been conducted so far on *shatavari* supplementation on the basis of body weight in dairy cattle. To fill up the gaps in knowledge, *Shatavari* herb was selected as feed supplement to investigate its effect on growth and sexual maturity of Sahiwal heifers with the specific objectives: (i) To study the effect of herbal feed supplement *shatavari* on growth performance of Sahiwal heifers and (ii) to study the effect of herbal feed supplement shatavari on sexual maturity of heifers

In the present study experimental Sahiwal heifers were divided into two groups with 8 animals in each on the basis of Having similar range of body weight and similar age group and were kept in separate loose housing system. The experimental period was six months started from September (2011) to February (2012). The feeding schedule from September – December was Concentrate + Maize + Jowar while during January – February. it was Concentrate +Oat +Berseem for both groups with additional supplementation of *Shatavari* (@ 150 mg/ kg body weight / day) in treatment group. the effect of herbal feed supplement Shatavari on growth performance and sexual maturity of Sahiwal heifers was observed every 15 days interval during experimental period.

5.1 Effect of *shatavari* supplementation on Dry matter intake (DMI)

5.1.1 The overall average values of DMI (kg/day) during six months of experimental period were 4.70±0.09 and 5.35±0.18 in control and treatment group, respectively.

5.1.2 The overall average value of DMI (kg/day) of treatment group was significant at 5% level of significance.

5.1.3 There was significant increase in dry matter intake (kg/day) in treatment group compared to control groups from 5th fortnight onward upto 13th fortnight.

5.1.4 The significant increase in overall dry matter intake (kg/day) in treatment group was 2.40 kg per 100 kg body weight which is under the standard value of 2.5 kg /100 kg body weight.

5.2 Effect of *shatavari* supplementation on body weight

5.2.1 The overall average values of body weight (kg) during six months of experimental period was 206.74 ± 3.46 and 222.68 ± 5.7 in control and treatment group, respectively

5.2.2 Increase in overall average values of body weight (kg) of treatment group was significant at 5% level of significance.

5.2.3 There was significant increase in body weight (kg) in treatment group compare to control groups from 7th fortnight onward upto 13th fortnight.

5.3 Effect of *shatavari* supplementation on average daily gain (gm/day)

5.3.1 The overall average values of growth rate (gm/day) during six months of experimental period was 223.26 ± 21.53 and 345.34 ± 23.40 in control and treatment group, respectively.

5.3.2 The overall average level of growth hormone (ng/ml) of treatment group was significant at 5% level of significance.

5.3.3 There was significant increase in growth rate (gm/day) in treatment group compare to control groups from 5th fortnight onward upto 12th fortnight.

5.4 Effect of *shatavari* supplementation on Growth hormone

5.4.1 The overall average level of growth hormone (ng/ml) of treatment group was significant at 5% level of significance.

5.4.2 The overall average level of growth hormone (ng/ml) during six months of experimental period was 5.41±0.15 and 6.33±0.11 in control and treatment group, respectively

5.4.3 There was significant high level of growth hormone (ng/ml) in treatment group compare to control groups from 7^{th} fortnight onward upto 12^{th} fortnight except at 9^{th} fortnight.

5.5 Effect of *shatavari* supplementation on Cortisol

5.5.1 The overall average level of cortisol (ng/ml) during six months of experimental period was 3.52±0.068 and 3.44±0.08 in control and treatment group,

5.5.2 There was no significant change observed in both control and treatment group in cortisol level.

5.6 Effect of *shatavari* supplementation on age at puberty

5.6.1 The average age at puberty of Sahiwal heifers in control and treatment group was 739.66±19.17 and 713.60±16.10 days.

5.6.2 The average age at puberty of Sahiwal heifers of treatment group was significant at 5% level of significance.

5.6.3 The progesterone level was < 1 ng/ml in both groups on date of first heat.

5.7 Effect of *shatavari* supplementation on age at first service

5.7.1 The average age at first service of Sahiwal heifers in control and treatment group was 846.10±24.0and 817.40±20.37 days, respectively

5.7.2 The percentage of first service was recorded in both control and treatment group 37.5 and 75 respectively.

CONCLUSIONS

Feed supplementation of *shatavari* @ 150 mg/ kg live body weight / animal/ day for six months in two different feeding schedule from September – December and January – February improved body weight, growth rate, dry matter intake and level growth hormone while supplementation of *shatavari* lowered age at puberty and age at first services in Sahiwal heifers.

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