

# Effect of supplementation by cactus (*Opuntia ficus indica f. inermis*) cladodes on reproductive response and some blood metabolites of female goat on pre-mating phase

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**Abstract** – The objective of this study is to investigate the effect of barley substitution by cactus during pre-mating phase on some reproductive parameters and blood metabolites of goats.

Forty adult goats of local breed were allocated to two groups based on live their weight. Each goat of both groups received 0.9 kg of barley straw. Goats on concentrate group (CC) received in addition 425g/goats/day of a concentrate (mixture of 80% barley and 20% soybean). Goats in the cactus group (CAC) received 256g of a concentrate (mixture of 68% barley and 32% soybean) and 2600g of cactus pads. Feeding regimes were applied on the average for one sexual cycle before mating and continued until 5 weeks after.

Cactus incorporation had no effects on live weight and blood metabolite levels (glucose and total proteins) of the two groups. But goats receiving barley had a higher ( $p < 0.05$ ) plasma concentrations of urea than those receiving cactus pads.

Reproductive parameters as estrus distribution, ovulation rate at first estrus ( $1.26 \pm 0.11$  vs  $1.37 \pm 0.11$  respectively for goats in CC and CAC groups) conception rate at all estrus (75% vs 90% respectively for goats in CC and CAC groups) and litter size for all estrus ( $1.46 \pm 0.13$  vs  $1.27 \pm 0.11$  respectively for goats in CC and CAC groups) were not significantly affected by the regimes. It is concluded that during autumn, the sexual season, cactus can replace barley on the diet of goats during 8 weeks around the breeding without any negative effects on live weight, metabolites and reproduction outcome.

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**Keywords:** goats, cactus, ovulation rate, calcium, reproductive parameters

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## 1. Introduction

Where feed is scarce and of low nutritive value larger animals are at a disadvantage because of their greater maintenance requirements. Smaller animals like the goat with lower maintenance needs can cover large areas to gather sufficient nutrients for survival and for minimum production of meat and milk (Shankarnarayan et al., 1985).

Like in other arid and semi-arid zone, goat farming in Tunisia is extensive. Half of the goat herds are found in arid and semi-arid regions and are generally conducted in mixed flocks with sheep. Goat herds derive more than 75% of their food resources from natural vegetation (Rekik et al., 2005).

In such low input system, the sole guarantor to avoid falling productivity in herd and led to a sustainable animal production is to reduce feed cost through identifying cheaper sources of feeds (Shalander and Roy, 2013). In the Mediterranean region, alternative feed resources can be native pastures or rangelands, crop residues mainly straw and stubbles or locally available agro-industrial by-products (Rekik et al., 2013). In comparison to the use of conventional feed particularly concentrates, the option of using alternative feed resources is more economic, more ecological as it integrates the animal to its environment and can improve soil and water conservation (Rekik et al., 2007; Ben Salem and Smith,

2008). A typical example of this type of vegetation is the spineless-cactus (*Opuntia ficus-indica f. inermis*) which is widespread in North Africa, Latin America and West Asia.

In Tunisia, many studies were based on the use of cactus cladodes as an economic supplement for sheep having very low-quality diets (Ben Salem et al., 2004). Cactus cladodes have a high proportion of water (850–900 g/kg) but, at the same time, a high energy-content, providing up to 700 g/kg dry matter (DM) of carbohydrates (Nefzaoui and Ben Salem, 2002).

Previous studies showed a positive effect of a short-term nutritional supply with cactus cladodes on follicle development and ovulation rate in sheep (Rekik et al., 2011). Incorporation in the diet of cactus cladodes at any stage of the reproductive function in Barbarine sheep, does not depress measured reproductive traits either in male or female (Sakly et al., 2013). Numerous trials have addressed to the effects of the incorporation of this shrub on the ingestion, digestibility of rations, growth of lambs and quality of meat. Cactus thanks to its high sugar content could replace partially or totally conventional feedstuffs either forages or concentrate feeds without reducing livestock performance of sheep and goats (Ben Salem et al., 2002; Ben Salem and Smith, 2008).

To our knowledge, there has been no work on the effect of cactus incorporation into the diet of females in the pre-mating phase as flushing on goats. The general objective assigned to this study is to investigate the effect of a substitution of barley grains by the cactus on reproductive parameters and some blood metabolite levels on goat.

## 2. Materials and Methods

### 2.1. Study location and animals

The experiment was carried out between the end of September and the end of November at the Institut National de la Recherche Agronomique de Tunis experimental station of Ouesslatia. This period represents the season when goat resumes sexual activity after a period of spring and summer anestrus (Lassoued et Rekik, 2005).

The region of Ouesslatia is located at 200 km southwest of Tunis (35°51' N and 9°35' E). It's a semi-arid zone with an average of rainfall of 400 mm characterized by its extremely irregular seasonal distribution.

### 2.2. Goats, treatments and health management

Forty Maure Tunisian goats were used for the experiment (mean live weight  $\pm$ s.d  $32 \pm 3.9$  kg and mean age  $\pm$ s.d  $4.6 \pm 2.43$  years). Animals were randomly allocated to two equal groups containing 20 females each. Before the application of the treatments, all goats were treated with two im dose of 250  $\mu$ g of cloprostenol 11 days apart (1 ml. Estrumae<sup>®</sup>, Schering-Plough, Animal Health U.K.) in order to induce Corpus Luteum regression and estrus synchronization. Application of the treatments started on the day of estrus detection (considered also as the day of buck introduction). Treatments consisted on concentrate-based diet for the control group (CC, 80% barley + 20% Soybean, 425g) or concentrate supplemented with cactus for the 2<sup>nd</sup> group (CAC, 68% barley + 32% soybean, 265g; cactus, 2600 g). Both groups received in addition 900g of barley straw. Cladodes of the spineless-cactus (*Opuntia ficus-indica f. inermis*) were cutted in small pieces by manual chopper and distributed to CAC group. Diets were iso-nitrogenated and isoenergetic and were calculated on the basis of the food tables of INRA (1988). Quantities formulated are assumed to cover 1.4 female maintenance requirements indicated to prepare goats for the breeding season.

At the start of the experiments mean Live weight and age in group CC and CAC were similar ( $31.6 \pm 0.88$ kg,  $3.93 \pm 2.05$  CC;  $33.1 \pm 0.89$ kg;  $4.57 \pm 2.13$  CAC, respectively).

During the experience animals of each group were housed together separated from the other group by a fence and isolated from bucks by at least 1.5 km. Fresh water was continuously available. Regimes were distributed daily in two equal meals at 9:00h and 16:00h.

Prior to the experiment, animals were drenched against internal parasites using Oxfendazol<sup>®</sup> (Medivet, Soliman, Tunisia) at a conventional dose of 5 mg/kg body weight and vaccinated against enterotoxaemia using Coglavax<sup>®</sup> (CEVA santé animale, France).

### 2.3. Reproduction management

Oestrus was detected by 5 entire and experimented Maure bucks twice a day (morning and afternoon) for one hour each time. When they showed oestrus, controlled mating was applied to the females of the two groups at the sex ratio of 1:8.

Twenty days after buck introduction, all goats were submitted to laparoscopy.

## 2.4. Measurements

For each experimental group, collective refusal remaining in feed bins the following morning were registered and weighted daily. Subsamples (20%) of feed offered and refused were collected once a week and stored at  $-5^{\circ}\text{C}$  pending analyses.

Live weight of fasted goats was measured once a week at 08 :00 h before meals distribution using livestock weigh scales with a capacity of 150 kg and an accuracy of 0.1 kg.

On day 4 after the start of mating, other blood samples were collected into evacuated tubes without additives for the assessment of blood metabolites.

After centrifugation (3000 rpm, 15mn), plasma and serum were collected and stored at ( $-18^{\circ}\text{C}$ ) until assayed.

The presence and number of corpora lutea of fasted goats, were assessed using laparoscopy (Thimonier and Mauleon, 1969).

## 2.5. Laboratory analysis

Feeds, collective refusals were analyzed for dry matter (DM), ash, organic matter (OM), crud protein (CP) according to standard procedures (AOAC, 1984) and Neutral Detergent Fiber (NDF) using the procedure described by Goering et Van Soest (1970). Oxalates were determined following the procedure described by Moir (1953).

Serum collected from blood samples were analyzed for urea, glucose, total protein, calcium and phosphorus. Serum levels of glucose, total protein, urea, Ca and P were all determined using commercially available kits (Biomaghreb®, Tunis, Tunisia). All kits were based on bio-chemically established procedures and concentrations were measured photometrically at specific wavelengths.

## 2.6. Statistical analyses

Data on blood metabolites concentrations was tested by the ANOVA procedure of the SAS software (SAS, 2005) with the diet as the only effect considered. When the diet was significant, mean treatment groups were compared according to the null hypothesis of the Least Square Means option. All results were expressed as mean  $\pm$  SEM (Standard Error of mean). Data on estrus distribution, conception rate and ovulation rate were analyzed using  $X^2$  test. The effect of the diet was considered significant when the level of probability was 5% or less.

## 3. Results

### 3.1. Feed composition

As expected, the analyses showed that cactus is rich on oxalate (14% of DM) (Table 1). Non-organic matter content of cactus (337g/kg MS) was low compared to concentrate while its water content was very high (expressed as the content of DM, 86%).

**Table 1.** Chemical Composition of feed

	Feeds			
	Barley straw	Cactus	Concentrate (CC groupe)	Concentrate (CAC group)
Dry matter (g/kg)	914	141	896	899
Organic matter (g/kg DM)	936	663	959	954
Crude protein (g/kg DM)	31	37	195	20
NDF(%DM)	68	25	28	25
Oxalates (%DM)	0.21	14	0.25	0.23

DM: dry matter; NDF: Neutral Detergent Fiber

### 3.2. Live weight

Mean Live weight in group CC and CAC was similar at the start of the experiment ( $31.6 \pm 0.88\text{kg}$ , CC;  $33.1 \pm 0.89\text{kg}$  CAC;  $p=0.225$ ). Nature of diets did not affect the evolution of live weight of goats in both groups but it appeared that after 20 days of application of the diets, there were tendencies for a

slight but non-significant difference in mean live weight between the two groups at day 10 ( $30.4 \pm 0.90$  kg CC,  $32.9 \pm 0.90$  kg CAC;  $p=0.059$ ), day 30 ( $31.0 \pm 0.84$  kg CC,  $33.1 \pm 0.84$  kg CAC;  $p=0.076$ ) and day 50 ( $31.1 \pm 0.86$  kg CC,  $33.5 \pm 0.83$  kg CAC;  $p=0.053$ ). Until the end of the experiment, after 50 days of the start of the experiment, goats in both groups maintained their initial weight since no difference was observed between mean weights at the start and the end of the experiment ( $p=0.69$ ) (Figure1).

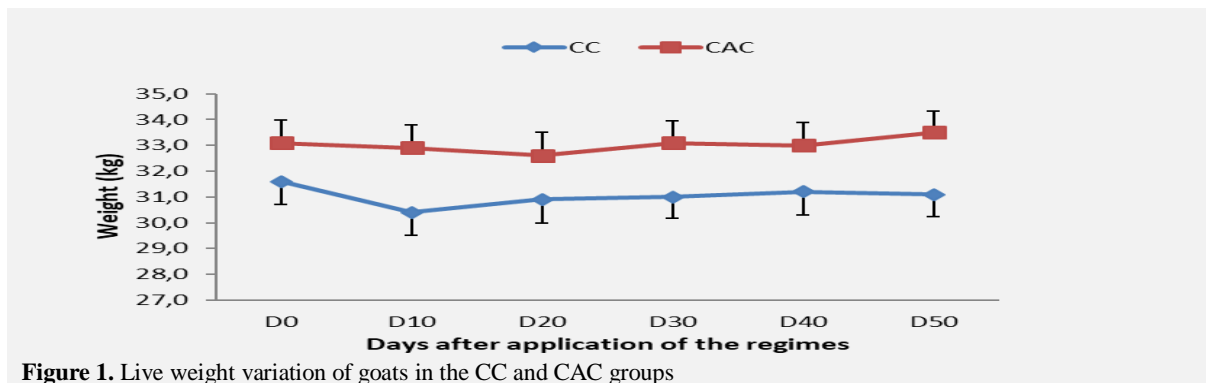


Figure 1. Live weight variation of goats in the CC and CAC groups

### 3.3. Blood metabolites and minerals

During mating period, there were no consistent differences between goats in the two groups in glucose, total protein, calcium and phosphorus concentrations ( $p>0.05$ ). However, urea concentration increased significantly on CC group compared to CAC group ( $p<0.0001$ , Table 2).

Table 2. Blood metabolites and minerals' concentrations ( $\pm$ SEM) of goats in CC and CAC groups

Parameters	Groups		Significance (P>F)
	CC	CAC	
Glucose (mmol/l)	2.69 $\pm$ 0.17	2.36 $\pm$ 0.17	ns
Total protein (g/l)	85.7 $\pm$ 3.33	82.5 $\pm$ 3.33	ns
Ureammol/l	9.27 $\pm$ 0.43	6.92 $\pm$ 0.43	***
Phosphorus (mmol/l)	1.82 $\pm$ 0.17	1.70 $\pm$ 0.17	ns
Calcium(mmol/l)	2.91 $\pm$ 0.15	3.24 $\pm$ 0.15	ns

(\*\*\*) : Significant at  $p<0.001$ , (ns) : not significant

### 3.4. Reproductive outcome

Percentage of goats showing oestrus each day is expressed in relation to the total number of goats put to mating on each group (Figure 2).

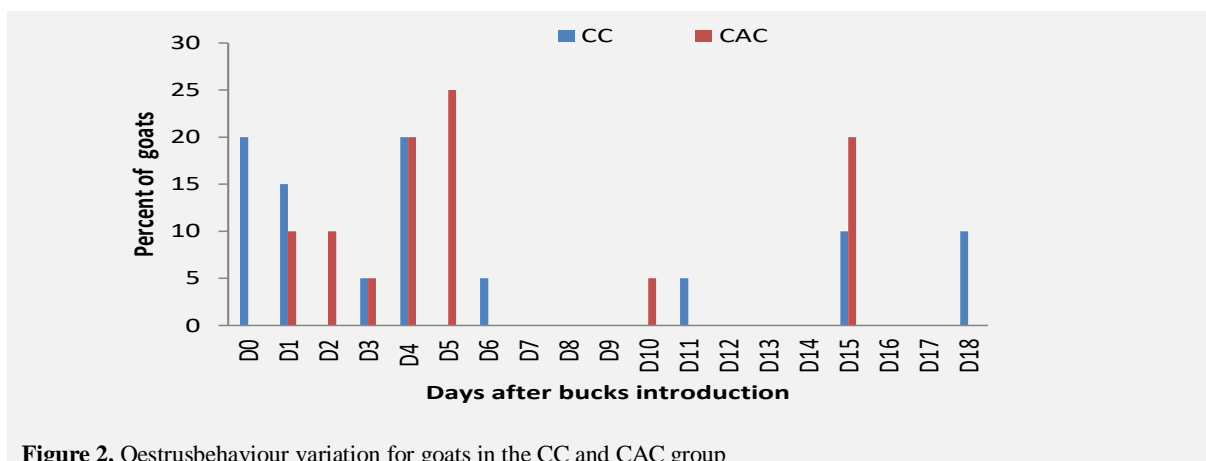


Figure 2. Oestrusbehaviour variation for goats in the CC and CAC group

All goats succeeded to exhibit oestrus excepting two female on CC group and one on CAC group. Heat distribution demonstrated that there were two main periods after buck introduction on which goats showed oestrus: from day 0 to day 6 and from day 10 to day 18. No statistical differences appeared between the two groups regarding oestrus behavior. Goats of the two groups were equally divided between those two periods of heat. In fact, from day 0 to day 6, 13 and 14 females came to oestrus respectively on CC and CAC group. From day 10 to day 18, 5 females came to oestrus on each group. Because of variability of response to cloprostenol synchronisation, we needed to calculate the duration of the application of the regimes in each group. Indeed, based on dates of kidding and fertile mating, the average within each group was  $22.5 + 3.05$  d and  $23.25 + 2.31$  d respectively for the CC and the CAC group.

Conception rate, considered as the per cent of ewes that lambed compared to the number of ewes exposed to the ram (Casas et al., 2004), was 75.5% and 90.0% respectively for CC and CAC groups. In both two groups, although this parameter was slightly high in the CAC group, the difference was not statistically significant (Table 3).

The proportion of goats that succeed to conceive after the first oestrus (induced by cloprostenol injection and buck introduction) was similar in the two groups. The figures were 45% and 75% respectively in CC and CAC group. The remaining others conceived at the 2nd and the 3rd oestrus (between 30 and 15%).

**Table 3.** Reproductive parameters for goat in CC and CAC groups\*

Parameters	Groups		Signification
	CC	CAC	
Conception rate (%)			
At first oestrus	45 (9/20)	75 (15/20)	NS
All oestrus	75 (15/20)	90 (18/20)	NS
Prolificacy (%) (all oestrus)	147 (22/15)	128 (23/18)	NS
Litter size (all oestrus)	1.46 ± 0.13	1.27 ± 0.11	NS
Ovulation rate (first oestrus)	1.26 ± 0.11	1.37 ± 0.11	NS

\* results are expressed as means ± SEM; NS: not significant  
 ‡ ( ) : number of observations

Litter size levels, after conception at all oestrus was statically similar between the two groups (Table 3). There was not any tendency for the ovulation rate recorded to differ between the two groups (Table 3).

## 4. Discussion

### 4.1. Chemical composition of diets

Cactus is increasingly being used as an alternative source of livestock feed in Tunisia. It's an interesting vegetable due the environmental conditions in which it grows and its resistance to climatic extremes. Nutritional quality of this forage depends on plant species, variety, clatode age, growing season and crop management. As reported by Nefzaoui and Ben Salem (1996), Ben Salem et al. (1994) in Tunisia or in many countries of south America (Azócar and Rojo, 2001; Lopez-Garcia et al., 2001), Cactus clatodes are high in moisture content (about 85 to 90% water), and low content of crude protein and crude fiber.

### 4.2. Live weight

Although it has been considered poor in terms of nutrients and fiber, cactus constitutes the main source of water in traditional production systems, particularly during the dry season. In this study the main objective was to substitute, in goats, the energy provided by barley by that provided by cactus in a classical flushing phase of 21 days. To control the period and the duration of application of the regime, the synchronization of the cycles was necessary. Average regime's application within each group was  $22.5 + 3.05$  d and  $23.25 + 2.31$  d respectively for the CC and the CAC group.

Even thought, globally we observed a same evolution of body weight between the two groups, there was a tendency for the goat on CAC group to be heavier than goats in CC group at day 10 ( $32 \pm 0.9$  vs  $30.4 \pm 0.9$ ,  $p=0.059$ ) and at day 50 ( $33.5 \pm 0.83$  vs  $31.1 \pm 0.86$ ). Live weight variation of the goats in CAC group reflects good palatability for this fodder and that the computed diets had a balanced and sufficient energy and nitrogen intake.

Several studies have highlighted the effect of cactus on growth and average live weight of small ruminants at different physiological stages. Atti et al. (2009) showed that in growing goat kids, after 12 weeks of experimentation period, live weight was similar for all groups that were subjected or not to the incorporation of cactus on their diets. Even more, cactus intake tended to increase the growth rate without significant difference between groups. Authors attribute this result to high energy and protein intake of groups fed cactus diets, which characterized by a high dry matter intake and digestibility. In the other hand, Ben Salem et al. (2002a; b) have shown that Barbarine ewes and rams fed a diet containing cactus lost weight compared to those receiving a barley diet. This is explained by the poverty of the cacti in nitrogen and / or its richness in oxalate.

#### **4.3. Blood metabolites**

Cactus intake did not impact blood metabolites since no significant differences were observed in the two groups regarding glucose, total protein, calcium and phosphorus concentrations during the period of flushing and mating. Besides, concentrations of glucose, total protein, urea, calcium and phosphorus in the serum of experimental goats were within the normal range reported by Jaziri (1985) for local goats.

Energy intake, a critical dietary factor impacting animal reproduction, was assessed by plasma glucose as indicative of the energy provided by the tissues (Scarmuzzi and Murray, 1994). Evolution of glucose concentration in the two groups showed that the energy status was the same in goats receiving concentrate or cactus in their diets. However, almost all carbohydrates in ruminants undergo fermentation to produce volatile fatty acids. Unfortunately, we did not estimate plasma concentration of non-esterified fatty acids presented as having closest relation with the intake of metabolisable energy, being capable in itself of indicating the animal's energy status (Fernandez et al., 2007).

Calcium concentration showed values that were within the range norms in goats (Jaziri, 1985). Values were slightly higher in the CAC group however; the difference was not statistically significant. Previous studies reported that the cactus is a calcium-rich plant compared to barley (56.4 g / kg DM versus 6.5 g / kg DM) and low in phosphorus and magnesium. Also, cactus has high level of oxalates considered as anti-nutritional substance (131 g / kg of MS for cactus and 3 g / kg of MS for barley) (Ben Salem et al. 2005). Oxalates are decarboxylated organic acids easily forming insoluble salts with calcium and magnesium (James, 1978). The anti-nutritional effect of this secondary compound has been demonstrated experimentally in vivo (James and Butcher, 1972) as in vitro (James et al., 1967). We expected that calcium concentration should be lower in CAC group in comparison to CC group due to the formation of oxalate-calcium complexes. This effect does not appear in our study. It is possible that the particular digestive characteristics of goat helped to cope with these secondary compounds. In fact, many studies have demonstrated that goats, especially those indigenous to woody areas are capable to consume much more browse sources rich on secondary compounds (i. etannins) than sheep and to digest it much more efficiently (Kumar and Vaithiyanathan, 1990; Silanikove et al., 1994, Silanikove et al., 1996). However, this major mineral is tightly regulated by homeostatic processes and its serum concentration may not reflect dietary intake (Muscher et al., 2008). Recent studies demonstrated that compared with sheep, goats have a greater ability to compensate for challenges to calcium homeostasis, probably due to a more pronounced increase in calcitriol production (Mirja et al., 2012).

Protein status is more difficult to establish. Combining measurement of total protein and urea may reflect protein imbalance or failure of renal function (Osbaldiston and Moore, 1971). Total protein and urea concentrations of the goats of the two groups were within the range of the goat reference values (Jaziri, 1985) during flushing period. It is concluded that cactus incorporation had no influence on renal function.

Total protein concentrations were not statistically affected by the diets; however, measurement of uremia showed a higher value on CC group in comparison to CAC group. This significant difference between the two groups may be simply due to a slightly unbalanced protein intake from barley and soybean meal and this might favor a decrease in protein catabolism for the CAC group.

#### **4.4. Reproductive outcome**

In this trial, results of heat detection showed no effect of the diet on this parameter. In fact, more than half of the goats in each group have externalized heat behavior within five days of buck introduction. Effect of supplementation on the reproductive performances of small ruminants is well documented (see review of Scaramuzzi et al., 2006). Such nutrient supplementation may be done with feeds high either in energy or in protein, using schemes that vary in timing and duration. Increasing energy intake to twice

maintenance requirements from day 2 to 14 post-mating resulted in a 25-30% reduction in embryo survival rate (Parr et al., 1987). Waghorn et al (1990) have shown that a diet with a high protein level, reflected by a high proportion of branched and circulating essential amino acids, also increases the ovulation rate. This finding is confirmed later by Downing and Scaramuzzi (1991) who showed that the ovulation rate increases after intravenous infusion of branched-chain amino acids. Following a synchronized oestrus, fecundity increased by 0.5/lamb/ewe when ewes were supplemented with 270 g soyabean meal/ewe/day for 14 days before and 2 days after mating (Molle et al., 1997). Regarding our experiment, cactus cladodes, although very low in protein, did not unbalance the ovulation rate. The values found are in the ovulation rate standards for the local goat at that time of the year (November-December). Indeed, Lassoued and Rekik (2005) found a rate of 1.42. This can be explained by the energy supply of the cactus, which is probably involved in the follicular development process and consequently on the ovulation rate. Rekik et al. (2008) showed that when ewes are supplemented in "foccus feeding" 6 to 9 days before mating with either concentrate or cactus, the number of large follicles is greater among sheep receiving cactus. In the same way, rate of immediate ovulation after rams' introduction is higher in ewes receiving cactus.

Conception rate obtained in this trial is comparable in both groups; however, a slight superiority in the CAC group was not statistically significant (90% vs 75% respectively for CAC and CC group). This may be due to the small number of females per group.

Short-term supplementation has no remarkable influence on the fertility rate, which depends more on longer-term or environmental and behavioral factors. In the Barbarine ewe exposed to 30 weeks under-feeding, fertility of the females was low resulting from a decrease in the ovulation rate and uterine receptivity even if these females were well fed during the month before mating (Lassoued, 1998).

Prolificacy and litter size, although higher in CC group, were not affected by the diet. Indeed, litter size of the goats receiving the cactus was smaller than those receiving barley. On a practical level and in addition to the studies made in sheep showing that cactus does not affect the reproductive performance of Barbary rams (Ben Attia, 2007) nor the performance of feeding and growth of young sheep Barbarine, this result is added to encourage the substitution of barley by the cactus as classic flushing period of 21 days.

## 5. Conclusion

Results of this study suggest that the cactus can replace conventional food resources such as barley in the diet without affecting live weight variation, reproductive performances or metabolic status. Indeed, using cactus as supplement of the ration would provide nutritional requirements of goats during breeding season, which is a critical physiological stage in the management of small ruminants.

Results obtained showed that the diet based on the cactus, in comparison with the conventional diet has no negative effect on concentrations of different metabolites. In fact, concentrations of phosphorus, total proteins, urea and glucose remain within the usual norms. As the serum calcium is high, the cactus, despite its high oxalate content, does not have an anti-nutritional effect and does not reduce animal's availability of calcium.

Also, this experience has shown that the substitution of barley by the cactus did not affect fertility or ovulation rate. However, these results deserve to be confirmed in future trials involving a larger number of females and with the possibility of using the cactus in focused feeding type supplementation.

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