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## Effect of body condition score on biochemical parameters and hormonal profiles during the mating period in Ouled Djellal ewes in arid zone

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**ABSTRACT:** This study aims to evaluate the effect of body condition score (BCS) on hormonal profiles and to reveal their influence on the blood metabolites in Ouled Djellal ewes during the mating period. The experiment was conducted on thirty, clinically healthy and non-pregnant Ouled Djellal ewes, which have been kept into two groups according to their body condition score (group I: BCS < 2.50 units; n = 13, group II: BCS > 2.50 units; n = 17). In both groups, oestrus was synchronized using hormonal patterns, then the ewes were left to be naturally inseminated. Blood samples were collected at the time of the sponge removal. Plasma concentrations of glucose, triglycerides, cholesterol, total proteins, albumin, creatinine, urea, Progesterone, triiodothyronine (T3) and Thyroxine (T4) were recorded. The plasma level of triglycerides and thyroid hormones (T3 and T4) increase significantly ( $P < 0.01$ ) in group II. Progesterone concentrations were similar ( $P \geq 0.05$ ) in both groups at mating period, although we recorded higher rate in group I. Therefore, we conclude that body condition score has a significant impact on some hormone profiles and blood parameters during the mating period in Ouled Djellal ewes.

**Keywords:** Body condition; Hormones; Metabolites; Mating period

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## INTRODUCTION

Research on rearing patterns has a long history. For many decades, ewes' productivity has been one of the main concerns of sheep farmers around the world (Parker and Pope, 1983). Its boost is an ultimate goal for the sheep meat production industry.

In Algeria, the sheep herd is a widespread animal resource. With over 26 million heads, breeding ewes represent nearly 80% of the total population (M.A.D.R., 2016). Sheep rearing has gradually broadened to cover the arid and semi-arid regions where Ouled Djellal breed, as the most emergent breed, is basically exploited for meat production (Taherti and Rachid, 2018). Widely considered to easily adapt to difficult environmental conditions, this breed enjoys good maternal skills and has fine quality of reproduction (Dekhili and Aggoun, 2007; Chellig, 1992). However, due to the archaic management of farms, the growth of the herds is still quite poor according to most professionals; especially with regard to the conduct of reproduction.

Improving the productivity of sheep farming goes hand in hand with the control of reproduction and the improvement of its performances. It allows choosing the period of farrowing, reducing the unproductive periods and optimizing the size of the embryo (Chemin-eau, 1996). Some authors advocate the genetic pathway and argue that it can be obtained by selection or by crossing. In this sense, new intensification techniques, especially in matters of reproduction control, such as the synchronization of oestrus using hormonal treatments have been introduced in the country. The reproduction control is expected to allow manipulating the reproduction periods according to the fodder availability and helps reduce the unproductive periods and to optimize the size of the embryo (Thibault and Levasseur, 1991). Although, some studies have indicated that the reproductive response of ewes subjected to this protocol can be influenced by several factors: the way breeding is carried out, duration and doses of Pregnant Mare Serum Gonadotropin (PMSG) (Quintero-Elisea et al., 2010), and genotype (Macías-Cruz et al., 2012). In particular, no study, to our knowledge, has clarified how Body Condition Score (BCS) during the mating period may affect the effectiveness of this hormonal treatment to improve the reproductive performance of Ouled Djellal ewes. In addition, reproductive performance is commonly correlated with blood metabolites and hormonal profiles, in this view, the formulated hypothesis is that BCS during

the mating periods affects blood metabolites, as well as hormonal profile, which in turn reduces the efficiency of estrus synchronization protocols to improve the reproductive performance of Ouled Djellal ewes. Therefore, the objective of this study was to evaluate the effects of BCS during mating period on biochemical parameters and on hormonal profiles (Progesterone, T3 and T4) in Ouled Djellal ewes.

## MATERIAL AND METHODS

### Experimental Zone

Our experiment was carried out at the Technological Institute for the Development of Saharan Agriculture (ITDAS), in the region of Biskra (34 ° 48 'lat. N. and 5 ° 39' long. E.). The Governorate of Biskra is located in the center-east of Algeria, at the gate of the Algerian Sahara. It is a real buffer zone between the North and the South, about 400 km southeast of the capital Algiers.

### Animals and management

Firstly, at the beginning of April, thirty clinically healthy, non-lactating, non-pregnant Ouled Djellal ewes, aged 2.5 to 5 years, having an average body condition score of  $2.8 \pm 0.9$  and weighing  $54.83 \pm 9.4$  kg were assigned into two groups of equal size according to their body condition score (group I: BCS < 2.50 units; n = 15, group II: BCS > 2.50 units; n = 15), using a scale from 1 (emaciated status) to 5 (extremely fat), assessed by the same well-experienced person.. Also, at least 70 days separated the date of lambing from the beginning day of the experiment. Rams are totally isolated from the females and are introduced into the herd only during periods of mate.

The ewes in our experience are reared in a semi-extensive system; are brought to the pasture twice a day (7 a.m. to 11 a.m. and 4 p.m. to 6 p.m.), and at night all ewes were housed in a barnyard. They receive fresh, clean water twice a day (one in the morning and one in the evening). All the ewes received the same daily supplementation of 200 to 300 g of commercial cereal concentrate and 300 to 400 g of good quality hay at the barnyard twice a day (at 12 p.m. and 8 p.m.). The amount of concentrate administered differed depending on the body condition score.

After one month, all the ewes had conserved the BCS level in group II. However, from ewes of group I that not conserved the target scores, two ewes were assigned to the group II as it was evident that these ewes could not sustain a low BCS.

### Synchronization of Oestrus

Oestrus cycles of the thirty ewes were synchronized during the first week of May (day 1), by the insertion of an intravaginal sponge containing 20 mg flugestone acetate (FGA) (CHRONOGEST®, MSD Santé Animale) and kept in for 14 days. After this period, at the day of sponge removal (day 14), ewes received an intramuscular (i.m.) injection of 400 IU of PMSG (Folligon®, MSD Santé Animale) to synchronize ovulation. The following day (day 15), the fertile ram will be introduced in each of the groups for natural mate (Thimonier et al., 2000). Then the ram was withdrawn 48 h after its first introduction (day 17).

### Blood samples

At the day of the sponge removal (day 14), a blood sample was taken from all the ewes of the study early in the morning. The blood samples (7 ml) were taken by puncture of the jugular vein. It was collected using disposable needles in vacutainer tubes with heparin anticoagulant, and centrifuged before exhaustion of 2 hours following the sampling at 3000 rpm at room temperature for 15 min. The plasma was divided into aliquots in microcentrifuge tubes. The collected plasma was immediately transported in a cooler to the laboratory and kept frozen at  $-20^{\circ}\text{C}$  till further analysis.

### Analysis method

The concentrations of plasma glucose, triglyceride, cholesterol, total proteins, albumin, urea and creatinine were determined using commercial kits. "SPINREACT, Spain", according to the standard method using a Spectrophotometer (UV) (UV-160A; Shimadzu Corporation, Japan). The principles of the

analytical methods, coefficient of variation and the references of the techniques used are grouped in Table 1.

Hormonal parameters Progesterone, T3 and T4 were estimated by radioimmunoassay (RIA) using a gamma counter (PC-RIA MAS; Stratec, Germany) employing RIA kits supplied by Tecan IBL International GmbH, Hamburg, Germany.

### Statistical analysis

Statistical analysis of the blood and hormonal variables was established using "IBM SPSS Statistics 20" software from SPSS Inc. Chicago, Illinois, USA. This software, allows the determination of the mean and standard deviation of each parameter, and the comparison of two means of each parameter between group I and group II using the Student's test (t-test). The differences were considered significant when  $P < 0.05$ .

### RESULTS

BCS influence on some biochemical and hormonal profile during mating period in Ouled Djellal ewes from arid area of central East Algeria are set out in Table 2. Mean plasma glucose and cholesterol of ewes in group I differed significantly ( $P < 0.05$ ) with ewes in group II. In parallel, a strong increase in the plasma triglycerides was recorded in ewes with BSC  $< 2.5$  (group II) ( $P < 0.01$ ). In addition, total proteins and plasma creatinine levels were higher in group II, but differences between the 2 groups were not significant. The plasma urea concentrations were higher in group I compared with in group II, plasma urea has not sig-

**Table 1.** Analytical methods

Parameter	Analytical method	Coefficients of variation (%)	References
Glucose	Trinder. GOD-POD	Intra = 0.54-0.52 Inter = 1.45-1.11	« SPINREACT » Réf : 1001191
Triglycerides	GPO-POD. Enzymatic Colorimetric	Intra = 0.39-0.43 Inter = 3.62 - 3.59	« SPINREACT » Réf : 1001312
Cholesterol	CHOD-POD. Enzymatic Colorimetric	Intra = 1.27 - 0.54 Inter = 2.14 - 1.24	« SPINREACT » Réf : 1001092
Total Proteins	Biuret. Colorimetric	Intra = 0.21 - 0.24 Inter = 1.05 - 0.94	« SPINREACT » Réf : 1001291
Creatinine	Modified JAFFE Method	1.75 - 3.97	« SPINREACT » Réf : 1001111
Urea	Bertholet. Enzymatic Colorimetric	Intra = 1.43 - 1.86 Inter = 2.33 - 1.96	« SPINREACT » Réf : 1001331
Albumin	Bromocresol green Colorimetric	Intra = 0.47 - 0.55 Inter = 6.20 - 5.90	« SPINREACT » Réf : 1001020

**Table 2.** Variation of biochemical parameters and hormonal profiles according to the BCS during mating period in Ouled Djellel ewe

Parameters	Group I (M±SD)	Group II (M±SD)	P value Group I Vs. Group II
Glucose (g/L)	0.49±0.36	0.42±0.25	<i>P</i> < 0.05
Cholesterol (g/L)	0.68±0.25	0.49±0.19	<i>P</i> < 0.05
Triglycerides (g/L)	0.27±0.1	0.35±0.03	<i>P</i> < 0.01
Total Proteins (g/L)	58.74±3.74	64.89±4.85	NS
Albumin (g/L)	25.71±2.31	28.89±4.57	<i>P</i> < 0.05
Creatinine (mg/L)	11.14±1.9	12.4±2.3	NS
Urea (g/L)	0.28±0.6	0.25±0.09	NS
T3(nmol/L)	1.90± 0.21	2.20±0.15	<i>P</i> < 0.01
T4 (nmol/L)	148.0±11.39	160.4±8.71	<i>P</i> < 0.01
Progesterone (ng/mL)	5.3±0.96	4.8±0.8	NS

Results are expressed as Mean (M) ±standard deviation (SD). NS=Non-significant

nificantly differed according to the BCS in ewes at mating period; however, the plasma albumin concentrations tended to be lowered in ewes group I compared to the ewes of group II (*P*< 0.05).

Moreover, BCS affected hormonal profile, thus the plasma T3 concentrations were the lowest recorded in group I (1.90±0.21 nmol/L) and the highest concentrations in group II (2.20±0.15 nmol/L) (*P*<0.01) as well, plasma T4 of group II is significantly higher than those in group I (*P*< 0.01). Additionally, although the mean Progesterone concentrations did not significantly differ between the 2 groups.

## DISCUSSION

In the present study, plasma glucose results for both groups fall within the range of international results (0.4 - 0.7 g/L). However, our results were superior to those obtained by (Yagoubi and Atti, 2020) (0.11 - 0.15 g/L), and lower than that obtained by (Carlos et al., 2015) (0.60 - 0.63 g/L) in ewes of the breed Morada Nova. In the present study, plasma glucose concentrations were significantly (*P*< 0.05) affected by BCS similar to the results of (Sitaresmi et al., 2020), unlike (Jalilian and Moeini, 2013) who found that ewes with different BCS did not affect glucose levels. However, (Caldeira et al., 2007) recorded a different metabolic status for ewes with different BCS with lower glycaemia for lean animals (BCS between 1 and 2) than for fatty animals (BCS between 3 and 4). In our study, glycaemia in group I was higher than that in group II, this hyperglycemia is probably due to the low insulinemia in group I, because their needs are higher than those provided by the ration, particular as regards energy during the mating period, animals will use their body reserves to compensate for the deficit,

unlike the ewes of group II, which did not mobilize their reserves sufficiently. More, animals with higher plasma glucose levels had more energy, which could be used for improving reproductive performance, as shown in previous studies (Yagoubi and Atti, 2020; Stefańska et al., 2016)

Cholesterol has an essential role: it acts as a precursor of steroid hormones and bile acids, as well as in the composition of cell membranes. The cholesterol concentrations in Ouled Djellel ewes are within the physiological standards reported by Macías-Cruz et al., (2017) (0.63g/L), but appeared relatively low compared to the data cited by Carlos, et al., (2015) (0.71 - 0.74g/L) and Jalilian and Moeini, (2013) (0.93 - 0.99 g/L). There is a significant increase (*P*<0.05) in cholesterol level in ewes with BCS < 2.5 in comparison with ewes with BCS > 2.5, this is consistent with the results obtained by (Özpinar and Firat, 2003). Thus, the increase in the value of cholesterol in group I, consequently leads to an increase in the concentrations of Progesterone during the luteal phase compared to group II (Deghnouche et al., 2019). In addition, the nutritional status of ewes around the mating period affects ovarian activity in several breeds of sheep; the energy deficit leads to the mobilization of lipid reserves and causes an increase in the concentration of cholesterol in group I. On the other hand, the attribution of these changes to variations in thyroid activity. Thyroid hormones stimulate cholesterol synthesis as well as the hepatic mechanisms that remove cholesterol from the circulation. The decrease in plasma cholesterol because the level of the last process exceeds that of the first. Plasma cholesterol concentration may be considered the most reliable indicator of dietary variation and indicates that blood chole-

terol is highly correlated with various dietary intakes (Boudebza, et al., 2016).

The highest plasma triglyceride (TG) concentrations were observed in group II and can probably be attributed to an increased rate of TG synthesis in the intestinal mucosa, due to increased availability of substrates (Caldeira, et al, 2007). In addition, (Mazur et al., 2009) showed lower values for plasma triglycerides in undernourished ewes. While, (Pesántez-Pacheco et al., 2019) reported that ewes with a higher BCS during the postpartum had a higher triglyceride concentration than sheep with a lower BCS. Thus, the low plasma triglyceride level in group I could be linked to a lack of glucose in the ration compared to group II (Herdt, 2000).

Total proteins measured in this study remains close to physiological standards cited by (Carlos et al., 2015) (60.2 - 68.4 g/L). However, they are higher than values reported by (Jalilian and Moeini, 2013) (47 - 55 g/L). From the results of the changes in proteinemia, no significant difference was recorded between the two groups. This is the same conclusion found by (Sitaresmi et al., 2020; Caldeira et al., 2007), total proteins was not affected by BCS in ewes. The level of total proteins varies in the same direction as the evolution of the body condition; lower in animals with a low body condition score, than in animals with a high BCS (Caldeira et al., 2007). The nutritional level and particularly the protein consumption is closely related to the BCS. Thus, this is probably related to the nutritional or health status of the animal, since the level of plasma proteins may reflect the nutritional or health status of the animal. All of these proteins, synthesized by the liver, also serve as hepatic markers. Indeed, the plasma concentration varies with the food intake; an increase in protein ingestion usually leads to an increase in protein synthesis at the body level, not to mention the protein synthesis, which is, reduced (group I) due to anabolic depression related to a hormonal upheaval.

Creatinine is formed by irreversible dehydration of creatine phosphate for the release of energy in skeletal muscles, and it is increased by the body's content of creatine, which is directly related to muscle mass and therefore to the BCS (Samra and Abcar, 2012). Plasma creatinine concentration was high in group II, these results agree with the results reported by Yagoubi and Atti, (2020) and Widiyono et al., (2020). Therefore, lower creatinine levels in group I was likely justified by low muscle mass and low protein turnover.

Albumin is a protein synthesized in the liver. It serves to maintain oncotic pressure, and transport thyroid hormones, fat-soluble hormones, free fatty acids, calcium, unconjugated bilirubin, and buffer the pH. In group II, the level of albuminemia is higher than that of group I, this significant difference ( $p < 0.05$ ) is probably related to the increase in calcium level due to the increase in parathyroid hormone (PTH) caused by estradiol, through hormonal activity, which requires high levels of albumin. (Schenck and Chew, 2010) has also been shown that hypoalbuminemia can cause a decrease in the amount of protein bound calcium and therefore possible total hypocalcemia and vice versa. In addition, this effect can be explained by the proportional availability of amino acids for albumin synthesis and by the role of albumin as a supplier of amino acids to the peripheral tissues when a nutritional shortage of protein occurs (Caldeira et al., 2007).

Uremia is a good indicator of nitrogen intake and assesses the level of nitrogen feed in ruminants (Titouine and Meziane, 2015). The indicated values of uremia are high in group I compared to group II. These values recorded in the two groups are in the reference intervals cited by Kaneko et al., (2008) (0.17-0.43g/L). A comparison of means did not show significant differences between the two experimental groups. This increase of uremia in Group I, which is undernourished ewes compared to Group II, can be explained by mechanisms adopted to conserve as much nitrogen as possible, increasing urea recycling in the rumen and decreasing its urinary excretion (Marini and Amburgh, 2003).

Progesterone is the main hormone involved in the maintenance of pregnancy and may play an important role in mediating the effect of nutrition on embryo development and that the high level of plasma progesterone over the luteal phase of the estrous cycle is an indicator of high conception rates (Safsaf et al., 2012). In addition, progesterone is a hormone, which regulates various reproductive functions. Progesterone plays a key role in regulating the length of the estrous cycle and implantation of blastocysts. Barnett et al., (1978) report that high levels of progesterone in the blood can improve ovulation rates, embryo survival and nesting of fertilized eggs in the uterus and consequently reproduction rates.. Ewes of group II had a decreased concentration of progesterone compared with ewes of group I. At the time of sponge withdrawal in ewes of group I, progesterone concentrations averaged  $5.3 \pm 0.93$  ng/mL, at same time in ewes of

Group II, progesterone concentrations averaged  $4.8 \pm 0.8$  ng/mL. Similarly, a previous study demonstrated that progesterone concentrations at the time of sponge removal averaged  $5.1 \pm 0.3$  ng/ml (Van Cleeff et al., 1998). In fact, progesterone exerts a negative feedback effect on the hypothalamus and pituitary to regulate gonadotropin release, mainly directed towards luteinizing hormone (LH) release (Dias et al., 2015). In group II, one possible explanation for this fact, the decrease in mean progesterone at the time of sponge removal would not be sufficient to suppress the hypothalamic-pituitary axis, leading to an estrus response (Moonmanee and Yammuen-art, 2015; Blaschi et al., 2014). Also, there was higher plasma progesterone concentration in group I than in Group II. This suggests that BCS around the mating period only slightly affects the circulating progesterone concentrations, but not so significantly. Thus, it is possible that progesterone levels in blood were a little increased with feed restriction before mating (Macías-Cruz et al., 2017; Abecia et al., 2006). As in our study, it is assumed that the distributed ration to the ewes of group I does not cover all their nutritional needs, because it is a ration that ensures the maintenance of the body condition score of thin ewes. On the other hand, the increases in plasma concentrations of progesterone are noted in response to feeding restriction because feed restriction may have altered the metabolic clearance of progesterone (Safsaf et al., 2012).

Both T4 and T3 followed a similar direction in the present study. Their level decreased in group I. In addition, blood thyroid hormones are considered to be good indicators of the nutritional status of an animal (Sejian et al., 2010). Following feed restriction or food deprivation, plasma thyroid hormone concentrations were reduced in sheep (Rae et al., 2002). These

effects suggest that energy balance could play a major role in affecting the decrease in plasma thyroid hormone levels. The decrease in thyroid hormones could be a response to negative energy balance. Nutrition plays a primary role in thyroid gland activity and in blood thyroid hormone concentrations (Stefańska et al., 2016; Sejian et al., 2010; Todini, 2007). Hence, nutrition restriction could have elicited a reduction in thyroid hormone concentration in group I.

## CONCLUSION

The analysis of the results showed that the body condition score of the ewes has an influence on glucose, cholesterol, triglyceride, albumin and thyroid hormones (T3 and T4); on the other hand, the plasma concentrations of the total proteins, creatinine, urea, and progesterone have no significant differences at the mating period.

Finally, to set reproductive standards and enrich knowledge on the effect of body condition scores on reproductive performance and on hormonal and biochemical parameters, other studies should be carried out to promote these findings.

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## CONFLICT OF INTEREST

All the authors declared they have no conflict of interest.

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