

Comparative analysis of blood protein and progesterone levels in crossbred goats and Damascus in Algeria's arid region

Análisis comparativo de los niveles de proteína sanguínea y progesterona en cabras cruzadas y cabras de Damasco en la región árida de Argelia

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ABSTRACT

The study evaluated the influence of breed and reproductive status on blood progesterone and protein profiles in a sample of ten does aged between 1.5 and 3 years. The female goats were categorized into two equal groups: the Damascus breed and a crossbred group (Damascus x Arbia). All subjects underwent estrus synchronization followed by natural mating. Blood samples were collected at various stages: before the introduction of intravaginal sponges, early pregnancy (30 days after sponge removal), mid-pregnancy (90 days), and late pregnancy (130 days), and the post-partum period at 30 days. The findings revealed a significant ($P < 0.01$, $P < 0.001$) impact of reproductive stage on progesterone levels in both groups, alongside notable albumin levels in the Damascus does ($P < 0.05$, $P < 0.01$). However, total protein and globulin concentrations did not show significant variations across the physiological phases in either group. Specific effects related to breed were detected in early and mid-pregnancy for progesterone, and in early pregnancy and lactation for total protein levels, and for globulin during lactation. The changes in progesterone and protein profiles across both groups suggest physiological adaptations in the goats to accommodate the increased demands of gestation and lactation in Algeria's arid environment.

Key words: Crossbred; Damascus; goat; progesterone; protein profile.

RESUMEN

El estudio evaluó la influencia de la raza y el estado reproductivo sobre los perfiles de progesterona y proteínas sanguíneas en una muestra de diez cabras de entre 1,5 y 3 años. Las cabras fueron categorizadas en dos grupos iguales: la raza Damasco y un grupomestizo (Damasco x Arbia). Todos los sujetos fueron sometidos a sincronización de estros seguida de monta natural. Se recogieron muestras de sangre en varias etapas: antes de la introducción de esponjas intravaginales, al principio de la gestación (30 días después del monta), a mitad de la gestación (90 días), al final de la preñez (130 días) y en el periodo posparto a los 30 días. Los resultados revelaron un impacto significativo ($P < 0,01$; $P < 0.001$) de la etapa productiva en los niveles de progesterona en ambos grupos, junto con niveles notables de albúmina en las cabras de Damasco ($P < 0,05$; $P < 0,01$). Sin embargo, las concentraciones de proteínas totales y globulina no mostraron variaciones significativas a lo largo de las fases fisiológicas en ninguno de los grupos. Se detectaron efectos específicos relacionados con la raza en el principio y medio de la preñez para la progesterona, y en el principio de la gestación y la lactancia para los niveles de proteína total y para la globulina durante la lactancia. Los cambios en los perfiles de progesterona y proteínas en ambos grupos sugieren adaptaciones fisiológicas en las cabras para compensar las mayores demandas de la gestación y lactancia en el entorno árido de Argelia.

Palabras clave: Mestizo; Damasco; cabra; progesterona; perfil proteico.

INTRODUCTION

Goats (*Capra hircus*) are probably the most well-adapted farm animals to difficult environmental conditions; their adaptable feeding habits and ability to utilize scarce vegetation add to goats' value in marginal areas [1].

The Algerian goat population increased by 56.7 % between 2000 and 2018. It passed from 3 million to 4.9 million heads [2]. It is mainly from four native breeds: Arbia (or Bedouine), Makatia (or Beldia), Kabyle Dwarf, and M'zabite [3]. The dairy European Saanen and Alpine breeds, introduced from Europe, are the main population of imported breeds. The introduction of the Damascus (Shami) breed, a native breed of Syria and other Near Eastern nations, to Algeria has occurred recently, accompanied by substantial import costs. This breed is distinguished by its high prolificacy and exceptional milk production, making it one of the foremost dual-purpose breeds in the Middle East, particularly effective in semi-intensive or intensive production environments [4].

Therefore, to elevate the quality of their herds, farmers frequently utilize crossbreeding techniques involving Damascus bucks. Recognized for their pronounced seasonal breeding patterns, Damascus goats begin their breeding season in late August and maintain this period until around mid-December [5]. The anestrus period for Arbia goats extends from mid-March until mid-July [6].

The productive capabilities of female goats are influenced by a combination of genetic, environmental, and physiological factors [7]. Assessing progesterone levels during different physiological stages is a critical measure for evaluating the fertility status of these animals [8]. It is a reliable method for estrus detection, early diagnosis of pregnancy, prediction of litter size and parturition date [9], post-partum resumption of ovarian activity [10], and determination of pregnancy failure [11].

Conversely, blood biochemical parameters offer valuable insights for assessing various aspects of productivity, metabolism, nutrition, health status, welfare, and animal physiology. They are important in diagnosing diseases, determining prognosis, and prophylactic programs [12, 13].

Nevertheless, Blood biochemical parameters are affected by several determinants, including gender, breed, age, physiological conditions (like lactation and pregnancy), dietary factors, health issues, stress, seasonal changes, and agricultural systems [14].

The current research aims to examine the fluctuations in blood progesterone (P₄) and protein profiles during different reproductive phases in Damascus and crossbred does (Damascus x Bedouin) reared in the arid regions of Algeria, where goats' production, like other ruminants, suffers from a deficiency in nutritional requirements.

MATERIALS AND METHODS

Study location

The study was conducted on a privately owned farm situated in El-Doucen, Ouled Djellal, an arid area in the south-eastern part of Algeria (latitude 34° 06' N; longitude 5° 01' E). This region is distinguished by its dry climate, characterized by low rainfall,

an average summer temperature of 42 °C, and dry grazing areas. The study was conducted from July 2023 to January 2024, covering the time before estrus synchronization, the complete duration of gestation, and a postpartum period of 30 days(d).

Design of experiments and Animals

Ten apparently healthy does, aged between 1.5 and 3 years and exhibiting a body condition score (BSC) ranging from 2.5 to 3.5 on a scale of 1 to 5, were maintained for a three-week acclimatization period to the natural field conditions.

The female goats were categorized into two groups based on breed, with Group 1 consisting of Damascus goats and Group 2 comprising crossbred goats (Damascus x Arbia), each group containing five individuals.

To synchronize estrous cycles, an intra-vaginal sponge impregnated with 20 mg of flurogestone acetate (Chronogest®, Intervet) was inserted for a duration of 11 d. One d prior to the removal of the sponge, each doe received an intramuscular injection of 400 IU eCG (Folligon®, Intervet). Subsequent to the device's withdrawal, the goats were given the opportunity to mate naturally with fertile Damascus bucks, with the mating day designated as d 0 of gestation.

The gestation period was monitored through the absence of estrus and was further confirmed via transabdominal ultrasonography (DramińskiiScan, Poland) conducted 45 d post-mating. The goats were raised under a semi-extensive farming system, grazing on natural pastures while also being provided with barley (*Hordeumvulgare*) grain, wheat bran, and barley straw. They had unrestricted access to mineral licks, and water was supplied once daily. Additionally, the goats were hand-milked once each d [15].

Blood sampling, Progesterone, and protein profile assays

Blood samples were aseptically obtained from experimental animals through jugular venipuncture using vacutainers devoid of anticoagulants before morning feeding. This collection occurred at five distinct time points: prior to estrus synchronization (in non-pregnant goats before mating), The periods of interest are categorized as follows: early pregnancy, taking place 30 d subsequent to sponge extraction; mid-pregnancy, which is marked at 90 d; late pregnancy, occurring at 130 d; and the post-kidding interval, noted 30 d after the birth of the offspring.

The serum was obtained through centrifugation (SIGMA 2K15, Germany) at 1500 G for a duration of 15 minutes (min), and was then preserved at -20 °C (Bosch 380 L, Germany) until the analyses of progesterone and biochemical parameters were carried out. The quantification of progesterone levels in serum samples was conducted utilizing a chemiluminescence immunoassay kit, following the manufacturer's guidelines (Progesterone II kit, Cobas®, Roche). The coefficients of variation for intra- and inter-assay were recorded at 0.02 and 0.1, respectively.

The concentrations of blood metabolites, including total proteins (TP) and albumin (ALB), were assessed through enzymatic colorimetric tests using commercial kits (Spinreact,

Spain) with an automated biochemical analyzer (Mindray BS 330E, Shenzhen, China). The globulin (GLO) concentration was derived by subtracting albumin levels from total protein concentrations.

Statistical analysis

The analysis of progesterone levels and various biochemical parameters across distinct reproductive stages in both Damascus and crossbred goats was reported as means \pm standard deviation (SD). The Shapiro-Wilk test was applied to evaluate the normality of the data distribution.

A two-way Analysis of Variance (ANOVA) was carried out to explore the impact of breed and reproductive status on progesterone concentrations and biochemical markers, while Tukey's post-hoc test was utilized to identify significant differences among the mean concentrations of the parameters under investigation [15]. All statistical analyses were performed using GraphPad Prism software (version 7.00), with a significance threshold set at $P < 0.05$.

RESULTS AND DISCUSSION

This study demonstrated the influence of breed and reproductive status on blood progesterone and protein profiles. TABLE I and II are the mean values (\pm SD) of circulating P₄ concentrations alongside protein profiles recorded during different physiological phases, including pre-mating, pregnancy, and lactation, for both crossbred goats and Damascus.

Progesterone profiles

The level of progesterone present in the serum P₄ (TABLE I) exhibited an upward trend throughout the gestational period, subsequently returning to baseline levels following parturition in both Damascus and crossbred goat females. Notably, peak concentrations were recorded during the later stages of gestation in crossbred does. A significant effect of physiological status was identified ($P < 0.01$, $P < 0.001$) when comparing the pre-mating phase with early lactation and various stages of pregnancy (early, mid, late) across both groups. Additionally, significant differences between the goat breeds were detected during the early ($P < 0.01$) and mid-gestation ($P < 0.001$) periods, similar to what was reported by Belkacem *et al.* [15].

During the pre-mating period prior to sponge insertion, mean serum P₄ concentrations were found to be at basal levels (less than 1 ng/mL) in both groups, which is consistent with previous studies indicating that the goats were likely in an anoestrous or early oestrus state. The observed increase in P₄ levels suggests that oestrus was effectively induced in the goat does at the commencement of the breeding season through the use of a 20 mg fluorogestone acetate intravaginal sponge, along with an intramuscular injection of 400 IU eCG administered 24 h before the sponge was removed. This approach has been reliably applied during the non-breeding season in Iraqi goats, as noted by Taklan *et al.* [16].

The increasing trend of P₄ with the advance of gestation observed in both groups and the decline to basal levels after kidding were reported earlier. According to Jarrell and Dziuk [17], the P₄ concentration in goats was influenced by the number of corpora lutea (CL) early in pregnancy (from day d 7 to d 25 of gestation) and related to the number of conceptuses on d 45.

As pregnancy advances, P₄ levels tend to be similar in female goats irrespective of the number of CL. In the later stages of pregnancy, P₄ concentrations are predominantly influenced by factors that are not related to the number of corpora lutea or the presence of fetuses [17]. The ovary is identified as the main site of P₄ production in pregnant goats. This suggests that the irregular fluctuations in progesterone levels reported in the literature during gestation may be linked to variations in the composition and functionality of the CL [18].

Salve *et al.* [19] also discussed the potential influence of luteotrophic factors present in allantoic fluid on P₄ production by the corpus luteum. Moreover, the current study confirmed the significant effect of gestational stage on circulating P₄ levels, a finding that has been previously documented by Sousa *et al.* [20] ($P < 0.01$) in Caninde and Moxoto goats.

In contrast, Kouri *et al.* [21] observed a distinct pattern of progesterone levels in pregnant goats in Algeria, noting lower concentrations during the second week (3.31 ± 0.37 ng/mL) compared to the findings of the current study involving Damascus and crossbred does. They registered a peak level on week 15 (12.23 ± 1.23 ng/mL), a gradual decline between weeks 15 and 20 then a sharp decrease two weeks before parturition.

According to Gaafar *et al.* [22], blood P₄ in Damascus goats rose from 4.6 ± 2.8 ng/mL in the initial two weeks of gestation to

TABLE I

The influence of reproductive status on serum progesterone (P₄) levels (mean \pm SD) in Damascus and Damascus x Arbia female goats

Group	S1 Premating	S2 Early pregnancy	S3 Mid-pregnancy	S4 Late pregnancy	S5 Early lactation
(P ₄) Unit: ng·mL ⁻¹	G1 (n = 5) 0.28 \pm 0.16 ^{d1}	G2 (n = 5) 0.42 \pm 0.18 ^{d1}	9.25 \pm 0.66 ^{c1}	15.84 \pm 1.60 ^{b1}	23.04 \pm 0.48 ^{a1}
		12.74 \pm 1.12 ^{c2}	21.28 \pm 0.38 ^{b2}	23.33 \pm 1.90 ^{a1}	0.58 \pm 0.28 ^{d1}
					0.50 \pm 0.16 ^{d1}

P₄: progesterone (ng mL⁻¹); G1: Damascus; G2: Damascus x Arbia; S1: pre-mating; S2: early gestation; S3: mid-gestation; S4: late gestation; S5: early lactation. Different letters in the same row show a significant difference $P < 0.01$. Different numbers in the same column show a significant difference $P < 0.01$.

24.5 ± 3.1 ng/mL by the twelfth week. Subsequently, this level declined until the time of kidding, reaching 0.8 ± 0.4 ng/mL.

Hussain [7] also noted that the highest P₄ levels were recorded at the fourth month of gestation (9.15 ± 0.33 ng/mL), and that there was an increasing trend in Shami pregnant does. In this study, both groups showed higher levels than those reported by Hussain [7] in all samples.

Subsequent to parturition, blood levels of P₄ returned to baseline as a consequence of the regression of the CL, aligning with earlier research [8, 21].

In this study, the marked disparity in P₄ levels between Damascus and crossbred females observed during the early and mid-gestation phases can likely be ascribed to breed differences, corroborating the results reported by Mmbengwa *et al.* [14] who highlighted notable variations both among and within goat breeds as well as their feeding practices. These results are contradicted by the experiments of Abd El-Hamid *et al.* [23], who noted no significant effect of breed. The difference between both groups could also be related to age and parity of females, or the litter size [7]. The larger variability of the data between studies and breeds could be attributed to the breeding season, the management and breeding system, the nutrition and the genotype [18], or the biological sample (plasma or serum), the size of the experimental sample, the sampling frequency, or differences in the P₄ assay methods.

Protein profiles

Serum total protein and globulin profiles in both groups and albumin in the crossbred group showed no significant effect of reproductive status, while albuminemia in Damascus does was significantly higher at mid-gestation compared to other stages of pregnancy and lactation. The breed effect was detected in early pregnancy and lactation for TP and in lactation for GLO, with lesser concentrations in Damascus females compared to the crossbred group (TABLE II).

Mean values of PT, ALB, and GLO in Damascus and crossbred goats are included in the reference interval of Al-Rukibat *et al.* [24]. In line with these results in both groups, Abdul-Rahaman *et al.* [25] reported no significant variation in the TP concentrations between the non-pregnant and pregnant does at various stages of gestation.

In contrast, Allaoua *et al.* [26] recorded a significant effect of reproductive status with higher levels of TP (P<0.05) at the lactation period compared to other stages. However, the non-significant difference of albuminemia and globulinemia between physiological stages agrees with the present findings of both groups for GLO and ALB in the crossbred group.

Kumar *et al.* [27] found no variations related to pregnancy in TP, ALB or GLO. Furthermore, other reports indicated significantly lower serum TP levels in goats after parturition compared with pregnant ones, which is in line with the results of the Damascus group, but the difference was not significant [28]. According to Balıkcı *et al.* [12], the magnitude of blood TP changes increased with litter size and increased nutrient demands of the fetuses.

Recorded serum albumin concentrations (TABLE II) were highest at the mid-stage of gestation in Damascus goats, while Abd El-Hamid *et al.* [23] registered the highest levels at late pregnancy compared with other gestation stages. Jimoh *et al.* [29] noted significantly (P < 0.05) higher ALB in dry and early gestation does than in mid-and late gestation does.

Other reports revealed statistically higher proteinemia and albuminemia in the blood of gravid does compared to the lactating ones [13]. Hypoalbuminemia in early lactation may be ascribed to a decrease in protein synthesis in the liver, which is due to fatty infiltration following lipomobilization in animals, or a decrease in the availability of amino acids that are used primarily to satisfy the mammary gland demand for amino acids and glucose [30].

Further, Akkaya *et al.* [31] described a gradual decrease of ALB in pregnant Saanen goats until parturition and a significant

TABLE II

The influence of reproductive status on serum protein metabolites levels (mean ± SD) in Damascus and Damascus Arbia female goats

	Group	S1 Premating	S2 Early pregnancy	S3 Mid-pregnancy	S4 Late pregnancy	S5 Early lactation
TP (g L ⁻¹)	G1	70.40 ± 3.44	69.40 ± 5.13 ^b	70.00 ± 4.18	70.40 ± 3.51	64.20 ± 3.27 ^b
	G2	75.40 ± 3.44	76.00 ± 1.23 ^a	75.00 ± 4.30	72.20 ± 4.21	71.40 ± 3.91 ^a
ALB (g L ⁻¹)	G1	29.68 ± 0.41 ^a	29.38 ± 1.14 ^b	32.60 ± 4.02 ^a	28.64 ± 1.94 ^b	28.72 ± 1.07 ^b
	G2	30.70 ± 1.00	30.90 ± 1.07	32.26 ± 1.38	30.20 ± 2.08	29.30 ± 0.85
GLO (g L ⁻¹)	G1	40.72 ± 3.58	40.02 ± 5.48	37.40 ± 3.32	41.76 ± 3.83	35.48 ± 2.94 ^b
	G2	44.70 ± 2.94	45.10 ± 1.88	42.74 ± 3.56	42.00 ± 3.93	42.10 ± 3.79 ^a

G1: Damascus, n= 5; G2: Damascus x Arbia: n= 5.; TP: total proteins; ALB: albumin; GLO: globulin; RS: Reproductive status; Bd:breed S1: pre mating; S2: early gestation; S3: mid-gestation; S4: late gestation; S5: early lactation. Different letters in the same column show a significant difference P < 0.01

increase one week after kidding. But no such observation was recorded in the present experiment for the crossbred group in agreement with Bamerny *et al.* [32] who revealed no effect of physiological condition on ALB levels, whereas a significant decrease was observed in late gestation and early lactation compared to mid-gestation in Damascus goats.

During the last trimester of pregnancy, the blood experiences a quick extraction of immunoglobulin, which corresponds with the onset of colostrum production in the mammary glands, may explain decreased total protein and albumin levels over pregnancy and lactation [12]. Unlike previous findings in goats with significantly higher globulin values in pregnant females, serum concentrations in the current study were not affected by the reproductive stage in both groups [33]. Furthermore, Allaoua and Mahdi [34] recorded a significant increase in plasma globulin levels during early and end lactation, compared to the period before gestation ($P < 0.01$) in Arbia goats. These findings are inconsistent with the GLO decline, although non-significant, observed after parturition in Damascus does in the present study. That fact could be due to the mammary glands' extraction of immunoglobulins from the plasma.

Variations of TP, ALB, and GLO profiles suggest greater metabolic demand of lactation in the Damascus group. Regarding the influence of the breed, Damascus goats have had statistically lower concentrations ($P < 0.05$) of blood TP in early gestation and early lactation and lower GLO in early lactation compared to crossbred does. Similar findings regarding TP, GLO but not ALB were reported by Njidda *et al.* [35] who found significant differences for total protein, albumin and globulin among the Nigerian Indigenous goat breeds (Kano Brown, Borno White, and Sokoto Red).

No significant difference in serum ALB levels was found between breeds across the different sampling time points, which is consistent with the findings of Mohammed *et al.* [36]. In the same vein, neither ALB nor globulin (GLO) concentrations in the blood showed meaningful differences between Damascus and crossbred females throughout pregnancy, pointing to a similar pattern of protein metabolism adaptation in both groups during gestation. That said, these results do not align with what Abd El-Hamid *et al.* [23] reported, as they found notably higher ALB levels ($P < 0.05$) and lower GLO concentrations ($P < 0.05$) in Damascus does when compared to Baladi ones.

Breed variations regarding the TP, ALB, and GLO levels were also shown in a previous experiment conducted in Egyptian goat breeds (Baladi, Damascus, Anglo-Nubian, Angora) by Anwar *et al.* [37]. Saber *et al.* [38] recorded no effect of breed and seasons on globulinemia of lactating dairy goats (Shami, Mixed boar, and Baladi) which disagrees with the present study where the Damascus group showed significantly lower levels than the crossbred one.

CONCLUSION

This study highlights the influence of both reproductive stage and breed on serum progesterone and protein profiles in Damascus and crossbred (Damascus \times Arbia) does reared in Algeria's arid regions. Progesterone levels followed the expected gestational pattern in both groups, with notable breed differences

emerging during early and mid-gestation. Protein metabolites remained largely within reference ranges, with only selective effects of reproductive status and breed detected, particularly during early pregnancy and lactation. Overall, these findings suggest that both groups undergo comparable physiological adaptations to cope with the metabolic demands of gestation and lactation under arid conditions, though breed-specific differences in certain metabolites deserve further investigation in larger experimental populations.

Author contribution statement

All authors participated in the data collection and analysis procedures, as well as in the writing of this article.

Conflict of interest statement

There were no reported conflicts of interest from the authors.

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