The Blood Biochemical Profiles in Algerian Rabbits of the Semi-arid Region of Aures (Algeria) At Different Physiological Stages

Moumen Souad, Belkadi Souhila1, Bouchareb Chahrazed2, Adjroud Hamida3

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Introduction

Blood parameters represent a great diagnostic tool for animal health status and disease evaluation (Argente et al., 2019; Barkakati et al., 2015). Hence, establishing their standard reference ranges become imperative for any disease diagnosis, prevention and controlling program as it forms the very basis for clinical interpretation of laboratory data (Moore et al., 2015). It is well known in rabbits that various factors as breed, gender (Tokarz-Depta et al., 2014), physiological condition (Tůmová et al., 2017) affect the metabolic profile (Moumen 2017; Zerrouki et al. 2009; Berchiche et al. 2000). Metabolites concentrations and fat mobilization affect the fertility rate (Brecchia et al. 2008). The nutritional status can be appreliended with the help of blood markers (Xiccato et al. 2004). The digestive and metabolic adaptations in response to under-nutrition has been described in many species including ruminants (Lamothe, 2003; Habeeb et al. 2019). However, the information is really scarce about plasmatic parameters of the Algerian population of rabbits reared in the region of Aures during their different physiological stages.

The aim of this study was to determine the levels of certain plasmatic biochemical values during different physiological stages of the local rabbit doe.

Materials and Methods

Animal source

One hundred and twenty (n=120) nulliparous does of local Algerian population were used for natural mating to study reproductive performance. All experimental rabbits were categorized as Empty(E), Pregnant (P), Pregnant lactating (PL) and Lactating (L). Does were first mated at the age of 16.5 weeks with an average body weight of 3107.1 ± 53.5g and with body condition scoring (BCS) of loin and rump ranging from 2 to 3 on a scale of 1-5 following a 42-day rhythm. Four blood samples were collected from females of five rabbits in each group viz. E, P, P-L and L respectively.

Result:

Milk yield differs significantly (p<0.05) between the two experimental groups (P-L and L). Litter size and litter weight of does at birthing was higher (p<0.01) in L group than in P and P-L groups. That the mean levels glucose in non-pregnant group recorded as 0.22 ± 0.04g/l which was increased 3rd week of pregnancy (0.65 ± 0.1 g/l followed by low mean value 0.35 ± 0.2g/l and 0.4+0.1g/l at pregnant lactating and lactating stages of experimental rabbits. The lowest level of protein was recorded in the second week of pregnancy (p<0.05). The increase in total protein, albumin and globulin concentrations in lactating group might be physiological. Concerning total lipids, there was a strong mobilization of body fat in pregnant females (97 mg/dl ± 0.10; p<0.05). However, a marked decrease (p<0.05) was recorded in cholesterolemia in lactating females. Regarding triglyceridemia, we have recorded a significant raise (37.7 ± 35.6; p<0.001) in pregnant females. The creatinine value recorded was close to the physiological norms 1.3-2.7 mg/dl vs 1.08-0.92 mg/dl. 73 plasma levels were lower (p<0.05) in lactating females’ group. Pregnant and lactating does showed an increase (p<0.05) of leptin level. The higher (p<0.05) progesterone levels in pregnant and pregnant-lactating does than its concentrations in empty and lactating does might be attributed to maintaining pregnancy. Therefore, the current study confirms that the analysis of metabolites represents a good tool to understand the physiological mechanisms.

Key words: Local population, Metabolic profile, Physiological status, Rabbit.
The Blood Biochemical Profiles in Algerian Rabbits of the Semi-arid Region of Aures (Algeria) at Different Physiological Stages (P-L) and Lactating (L). The trial was carried out at the experimental rabbit farm of the Veterinary Department of the University Batna 1 and last for a year (2016-06 to 2017-06).

Does were first mated at the age of 16.5 weeks with an average body weight of 3107.1 ± 53.5g and body condition scoring (BCS) of loin and rump (Bonnano et al. 2005) ranging from 2 to 3 on a scale of 1-5 following a 42-day rhythm. All does were kept in single cage (400x600x350 mm) made of galvanized wire. A female was eliminated after three successive refusals. Pregnancy diagnosis was done by abdominal palpation. Hence, all 120 experimental animals were grouped into four equal groups with 30 replicates (empty female, pregnant female, pregnant-lactating female and lactating female only).

A commercial pellet and water from nipple drinkers were provided ad libitum, the diet formulation and analytical data are presented in (Table 1). Chemical analysis was done according to AOAC procedures (2000). The light schedule of 16L/8D was used.

Biochemical analysis: Four blood samples were collected from females of five rabbits in each group viz. E, P, P-L and L respectively at: 08:00h, from the marginal ear vein, drawn into vacutainer tubes containing 0.85 U. I of Heparin, immediately centrifuged at 2000 x g for 10 mn and plasma stored frozen until assayed for hormones and metabolites. Plasmatic total proteins, albumin, total lipids, triglyceride and total cholesterol concentrations were determined colorimetrically using commercial kits produced by Stanbio Company, USA by computerized spectrophotometer model Milton Roy 1201. Plasmatic Globulin values were calculated by subtracting albumin values from their corresponding total proteins values of the same sample. Insulin, leptin, total triiodothyronine (T3) and progesterone (P4) hormones were determined using radioimmunoassay (RIA), by commercial Kit produced by IZOTOP Company (INSTITUTE OF ISOTOPES Ltd.) Hungarian Company (http://www.izotop.hu) and counting using a computerized gamma counter (ISOCOMP1–MGM) at the research laboratory of Pharmaceutical Mineral Chemistry.

### Statistical study

The results were expressed as mean and standard deviation (X ± SD). They were subjected to statistical analysis with one-way analysis of variance (ANOVA) test. All statistical analyses were performed using statistical software MedCalc version 15.2. A p<0.05 was considered statistically significant.

### RESULTS AND DISCUSSION

#### Reproductive performances

During pregnancy and suckling period, females had similar body weight but milk yield differs significantly (p<0.05) between the two experimental groups (P-L and L). Litter size and litter weight of does at birthing was higher (p<0.01) in L group than in P and P-L groups.

The improvement in does weight during pregnancy and suckling period might be due to the increased appetite of rabbits and consequently increased feed intake and good absorption in the intestinal tract Tůmová et al. (2017). Kids born of rabbits does of P and L groups weighed more and

#### Table 1: Formulation and chemical composition of diet.

| Ingredients     | %  |
|-----------------|----|
| Fat (%)         | 4.2|
| Starch (%)      | 14.0|
| Crude protein (%)| 17.2|
| Crude fiber (%) | 16.5|
| DE (kcal/kg)*   | 2440|

*Estimated according to Meartens et al. (1988).

#### Table 2: Reproductive performances of rabbit does at different physiological stages (n=30/group; Mean ± SD).

| Physiological status | E          | P          | P-L         | L          |
|----------------------|------------|------------|-------------|------------|
| Receptivity (%)      | -          | 55.5       | 58          | 64.7       |
| Fertility (%)        | -          | 51.2       | 45.5        | 59.4       |
| Initial BW (g)       | 3000 ± 37  | 3053 ± 47  | 3188.4 ± 63 | 3187 ± 67  |
| Cumulative BCS       | 3.00       | 2.2        | 1.96        | 2.56       |
| BW at kindling (g)   | -          | 2839.3 ± 70| 2703.3 ± 68| 3050 ± 57  |
| Milk yield (g)       | -          | -          | 435.1 ± 96  | 631.4 ± 136|
| - at 1° week         | -          | -          | 850.6 ± 37  | 1316 ± 156 |
| - at 2° week         | -          | -          | 935 ± 67    | 966 ± 155  |
| - at 3° week         | -          | -          | 2200.7 ± 36 | 2913.4 ± 105|
| Average MY during suckling period | -      | 20.9 ± 3.2     | 7.4 ± 2.37 | 9 ± 1       |
| LS at birth          | -          | 6.9 ± 2.3   | 7.4 ± 2.37  | 9 ± 1       |
| LW at birth (g)      | -          | 390.5 ± 13.6| 402 ± 11    | 501 ± 9    |
| LS at weaning (g)    | -          | 5.2 ± 2.3   | 5 ± 1.2     | 8 ± 2       |
| LW at weaning (g)    | -          | 2040 ± 67   | 2200 ± 25   | 3720 ± 39  |

BW: body weight, BCS: body condition score, MY: milk yield. **P<0.01; *P<0.05.
were of greater length than from does of pregnant-lactating group Rebollar et al. (2014). The difference between doe milk yields between experimental groups might be due to differences in litter size (Table 2). The increase in litter size and consequently litter weight of does at birth might be due to the high number of ovulation and fertility in female rabbits of the pregnant group of rabbits Zerrouki et al. (2009) and Theau-clement et al. (2013).

Biochemistry values in experimental groups

Glycemia can be a good reflection of the energetic balance of animals Habeeb et al. (2019). In rabbits, many authors observed a decrease in glycemia during pregnancy in response to the progressive increase of fetal growth needs. Blood glucose values are again very low in P-L female rabbits Fortun (2006) since the mammary gland is also a glucose sensor for the synthesis of milk lipids.

It was observed that the mean levels glucose in empty or non-pregnant group of animal of this study recorded as 0.22±0.04 g/l which was increased 3\textsuperscript{rd} week of pregnancy (0.65± 0.1 g/l) followed by low mean level 0.35±0.2 g/l and 0.4±0.1 g/l at pregnant lactating and lactating stages of experimental rabbits (Table 3).

Our results are in line with those of Chiericcato et al. (2004) on the Grimaud genotype but are above the values declared by Othmani et al. (2005) on local female rabbit breeds reared in the North of Algeria weighting 1988 g ± 234.19 (0.1 g/l vs 0.4 g/l) on average. However, our results remain below the values of the European rabbits and the rabbits reared in tropical areas 0.79 g/l Founzégué et al. (2007).

The lowest level of protein was recorded in the second week of pregnancy (p<0.05), a slight increase was then noticed. However, it ended by decreasing at the end of pregnancy. El Maghrawi et al. (2000) reported a significant raise in total proteins in the first half of pregnancy then it decreased at the end. Othmani et al. (2005) reported a higher level of protein (6.2 g/dl ± 4 vs 4 g/dl ± 0.90) in pregnant females. This parameter was the lowest in empty females (3.6 g/dl ± 0.5; p<0.05). The increase in total protein, albumin and globulin concentrations in does of lactating group of experimental rabbits might be might be physiological.

Immunoglobulin is the main component of antibodies, and increase in the immunoglobulin level indicates a good immune status of the animal Ballou et al. (2009).

Concerning total lipids, there was a strong mobilization of body fat in pregnant females (97 mg/dl ± 0.10). This significantly higher total plasma levels of lipids (p<0.05) could be explained by the energy needs that were more significant during pregnancy and lactation.

Regarding cholesterolemia, a highly significant increase (p<0.05) was observed in pregnant females. However, a marked decrease (p<0.05) was recorded in cholesterolemia in lactating females. This decrease can be explained by the use of cholesterol in the synthesis of steroid hormones. Same changes were observed in other species and in rabbits Chiericcato and Rizzi (1999). Regarding triglyceridemia, we have recorded a significant raise (37.7 ± 35.6; p=0.001) in pregnant females, which is in line with the results of Othmani et al. (2005). Hydrolysis by lipoprotein-lipase of triglycerides circulating in free fatty acids captured by underlying tissues, particularly the muscle, was considered as a limiting step for the use of triglycerides for energy purposes Hocquette et al. (2000).

In general, the circulating level of triglycerides diminished at the beginning of pregnancy when the metabolism was directed toward the storage of energy and increases at the end of pregnancy when the adipose tissues was mobilized.

The increase in total lipids, triglycerides and total cholesterol concentrations in pregnant does of might be due to the mobilization of body reserves to the needs of fetal growth and hormone synthesis. El Moghazi et al. (2014) indicated a decrease in total lipids, cholesterol and

**Table 3**: Effect of the physiological stage on metabolic profile and hormones in doe rabbits (n=20).

| Parameters        | E (n=5)   | P (n=5)   | P-L (n=5) | L (n=5)   |
|-------------------|----------|----------|----------|----------|
| Total proteins (g/dl) | 3.60 ± 0.5 c | 4 ± 0.90 b | 5.70 ± 1 a | 6.10 ± 0.1 a |
| Albumin (g/dl)   | 2.01 ± 0.1 c | 2.81 ± 0.05 b | 2.98 ± 0.07 a | 3.50 ± 0.10 a |
| Globulin (g/dl)  | 1.22 ± 0.02 c | 1.69 ± 0.01 b | 1.88 ± 0.06 a | 2.80 ± 0.04 a |
| Total lipids (mg/dl) | 31 ± 1 ± d | 97 ± 0.10 c | 69 ± 0.2 b | 78.01 ± 0.6 a |
| Triglycerides (mg/dl) | 15.4 ± 5.8 d | 65.80 ± 23 c | 44.7 ± 17.6 b | 37.7 ± 25.6 a |
| Cholesterol (mg/dl) | 28.7 ± 16.7 d | 70 ± 18.2 c | 58.3 ± 24.1 b | 38.8 ± 23.9 a |
| Uric acid (mg/dl) | 7.9 ± 1.4 d | 21.2 ± 3.8 c | 34.8 ± 3.4 b | 24 ± 3.3 a |
| Creatinine (mg/dl) | 0.72 ± 1.1 b | 1.4 ± 0.4 a | 1.3 ± 0.4 a | 1.2 ± 0.8 a |
| T3 (ng/dl)       | 22.4 ± 0.53 c | 31.6 ± 0.6 a | 44.3 ± 0.93 b | 29.2 ± 0.6 a |
| Progesterone (ng/ml) | 0.35 ± 4.2 c | 20 ± 0.2 b | 18.98 ± 1.5 b | 10.7 ± 0.15 a |
| Leptin (ng/ml)   | 1.5 ± 0.1 a | 2.50 ± 0.2 b | 1.7 ± 0.3 a | 2.02 ± 0.9 b |
| Insulin (µg/ml)  | 22.2 ± 1.2 a | 45.5 ± 2 b | 41.08 ± 3.2 b | 28 ± 1.02 c |
| Glucose (g/l)    | 0.22 ± 0.04 c | 0.65 ± 0.1 b | 0.35 ± 0.2 a | 0.4 ± 0.1 a |

a,b,c,d. Means in the same row within each physiological status having different superscripts differ significantly at P<0.05.
triglycerides in Californian pregnant and lactating rabbit does. The hypolipidemia in pregnant and lactating females might be due to suppression of hepatic lipogenesis Sampath and Ntambi (2005). The higher total plasma levels of lipids in does during pregnancy than its concentrations during suckling period might be due to increase in energy requirements of offspring and in the same time increased used these components in milk synthesis during the suckling period.

Creatinine and urea are two small molecules eliminated by the kidney of mammals. If creatinine is relatively constant in an individual but varies across breeds according to the muscular mass. Uremia, however, can vary in function of extra-renal factors (protein intake and liver functioning). The creatinine value recorded was close to the physiological norms 1.3-2.7 mg/dl vs 1.08-0.92 mg/dl Farougou et al. (2007). A low uremia level was noticed compared to the physiological norms, which range between 7.9-34.8 mg/dl vs 25.48-71.41 mg/dl Burke (1994). This low rate can be explained by insufficient protein intake to meet the needs of pregnant and lactating females. The lower uric acid and creatinine concentrations in empty rabbit does group than concentrations during pregnancy and suckling period might be increase these excretions from empty rabbits Salman (2017).

The T3 plasma levels were lower (p<0.05) in lactating females’ group. Brecchia et al. 2008 found that T3 blood concentrations decreased during fasting. Thus, the thyroid hormone concentration clearly reflects the energetic balance of the doe, and food deficiency reduced thyroid function, so that the animals could spare energy by decreasing adaptive thermogenesis Brecchia et al. (2008). Vanderpas (2006) concluded that the increase in basal metabolic rate is accompanied by increased appetite and subsequently increased body weight.

Insulin rate, which promotes the use of glucose by adipose tissue and thus the storage of energy under the form of fats, increases greatly at the end of pregnancy (Bracchia et al. 2008).

Insulin controls intermediate metabolism and exerts an important role in ovarian function as well. Since there is evidence of active transfer of both insulin and leptin into the brain, these hormones could have a role in signaling the metabolism state of the animal Woods et al. (2003).

Pregnant and lactating does showed an increase (p<0.05) of leptin level. Leptin receptors were detected in different structure of rabbits, including follicles at different stage of development and oviducts Zerani et al. (2005), suggesting that leptin may regulate steroidogenesis of pre-and post-ovulatory follicles as well as fertilization and early embryonic development by proving a favorable local environment to gamete (sperm and oocyste) Bolti (2004).

The higher (p<0.05) progesterone levels in pregnant and pregnant-lactating does than its concentrations in empty and lactating does might be attributed to maintaining pregnancy.

CONCLUSION

Both BCS evaluation, metabolites and hormonal analysis permit to manage properly the energy balance and to improve body status, reproductive performance and welfare of rabbit does. Naturally, this preliminary approach must be further studied, perhaps simplified and tested with a large livestock. Metabolites analysis represents a good tool for understanding the physiological mechanisms required to meet these objectives.

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