THE SERUM BIOCHEMICAL PROFILE OF THE RABBIT OF THE ALGERIAN POPULATION IN THE SEMI-ARID REGIONS OF AURES (BATNA) AT DIFFERENT PHYSIOLOGICAL STAGES

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Abstract: One hundred and twenty local Algerian female rabbits in first parity were used in this present study at the experiment farm of the University of Batna 1. The animals were divided into four equal groups with 30 replicates (empty female, pregnant female, pregnant-lactating female and lactating female only). Natural mating was used, the kits were weaned at 28 days. Four blood samples were collected on females at different physiological stages. The physiological status affected significantly ($p < 0.05$) cumulative BCS, milk yield, litter size and litter weight of does at birthing. Total protein, albumin, globulin, uric acid and creatinine concentrations as well as T3 and progesterone levels were higher ($p < 0.05$) in does of P-L group (5.70±1 g/dl, 2.98±0.07 g/dl, 1.88±0.06 g/dl, 34.9±3.4 mg/dl, 1.3±0.4 mg/dl, 44.3±0.93 ng/dl, 18.98±1.5ng/ml) respectively.

The physiological status increased significantly ($p < 0.05$) total lipids, triglycerides and total cholesterol in does of P, P-L and L groups compared with their concentrations in E group.
The physiological status affected alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase activities in does during pregnancy and suckling period (5.65±0.1UI/L, 22±1.3UI/L vs 5.45 ±0.22, 20.35±4.3UI/L).
Does of P, P-L, and L groups had higher ($p < 0.05$) leptin levels. Insulin and glucose concentrations were increased in P and P-L groups compared to E and L groups.

Key words: local population, semi-arid area, Rabbit, metabolic profile, physiological status.

Résumé : Cent vingt-neuf lapines de population locale algérienne de première parité ont été utilisées dans la présente étude à la ferme expérimentale de l'université de Batna 1. Les animaux ont été divisés en quatre groupes égaux comportant 30 sujets (femelle vide, femelle gestant, femelle gestant et allaitante et femelle en lactation seulement). L'accouplement naturel a été utilisé, les lapereaux ont été sevrés à 28 jours. Quatre échantillons de sang ont été prélevés sur des femelles à différents stades physiologiques. L'état physiologique a eu une incidence importante ($p < 0,05$) sur le BCS cumulé, la production laitière, la taille de la portée et le poids de la portée à la naissance. Les concentrations de protéines totales, albumine, globuline, acide urique et créatinine ainsi que les taux de T3 et de progestérone étaient plus élevés ($p < 0,05$) chez le groupe PL (5,70 ± 1 g / dl, 2,98 ± 0,07 g / dl, 1,88 ± 0,06 g / dl, 34,9 ± 3,4 mg / dl, 1,3 ± 0,4 mg / dl, 44,3 ± 0,93 ng / dl, 18,98 ± 1,5 ng / ml) respectivement.

L'état physiologique a augmenté de manière significative ($p < 0,05$) les lipides totaux, les triglycérides et le cholestérol total dans les dosages des groupes P, P-L et L par rapport à leurs concentrations dans le groupe E.
L'état physiologique de l'alanine aminotransférase (ALT), de l'aspartate aminotransférase (AST) et de la phosphatase alcaline a été affecté pendant la grossesse et la période d'allaitement (5,65 ± 0,1 UI / L, 22 ± 1,3 UI / L vs 5,45 ± 0,22, 20,35 ± 4,3 UI / L).
Les groupes P, P-L et L'ont des taux de leptine plus élevés ($p < 0,05$). Les concentrations d'insuline et de glucose ont augmenté dans les groupes P et P-L par rapport aux groupes E et L.

Mots-clés : Population locale, région semi-aride, lapin, profil métabolique, stade physiologique.

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INTRODUCTION

In recent years, several works have examined the reproductive traits of Algerian local population does (Moumen 2017, Zerrouki et al., 2009; 2005a; 2002; 2001;Berchiche et al., 2000) but no standardization study has been done on the plasmatic parameters of the Algerian population of rabbits reared in the Aures region during their different physiological stages.

The boy condition and energy balance of female rabbits appears to be correlated to short and long-term reproductive efficiency. Metabolites concentrations and fat mobilization also affect the fertility rate (Brecchia et al., 2008). The nutritional status can also be apprehended 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; Belkacem L et al., 2018; Habeeb et al., 2019).

The objective of this article is to examine some blood parameters at different physiological stages as specific markers of the body status.

I. Materials and Methods

I.1. Animals and experimental design

The trial was carried out at the experimental rabbit farm of the Veterinary Department of the University Batna 1. 120 nulliparous does of local Algerian population (Aures region) were submitted to natural mating. 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 (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 (400 x 600 x 350 mm) made of galvanized wire. Sexual receptivity was evaluated before mating and does with red and turgescent vulva considered receptive following the IRRG recommendation (2005).The females that refused to mate were presented to the males the next day. A female was eliminated after three successive refusals. A pregnancy diagnosis by abdominal palpation was performed 10 days after the mating. Non-pregnant females were presented to the males on the same day. The nest-boxes were placed three days before the presumed date of parturition. They were fixed outside on the cages.

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 environment temperature and relative humidity were collected (ranges: 15-20°C and 60-75%, respectively) and light schedule of 16L/8D was used. The building was artificially ventilated (0.3m^3/s).

I.2. Blood samples and estimation of biochemical components

Four blood samples were collected from all females at: 08:00h, at different physiological stages (empty female, pregnant female, pregnant-lactating female and lactating female only) from the marginal ear vein ,drawn into vacutainer tubes containing 0.85 U.I of Heparin, immediately centrifuged at2000 x g for 10 mn (SIGMA-2K15 centrifuge) and plasma stored frozen until assayed for hormones and metabolites. Serum total proteins, albumin, total lipids, triglyceride and total cholesterol concentrations as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase activities were determined calorimetrically using commercial kits produced by Stanbio Company, USA by computerized spectrophotometer model Milton Roy 1201. Serum 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), as reported elsewhere (Rommerset *al*., 2004) bycommercial 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.

I.3. Statistical analysis

The statistical analysis was performed with MedCalc version 15.2.1 according to the following model: $Y_{ij}= \mu \pm T_i \pm e_{ij}$where μ = the overall mean, Ti = the fixed effect of treatments, (1 = empty female, 2 = pregnant female, 3 = pregnant-lactating female, 4 = lactating female), e_{ij} = residual error. Least significant difference was used to compare between means.

II. Results

II.1. Reproductive performance

Compared to the does of P and P-L group, the does of L group had higher ($p<0.05$) sexual receptivity and fertility rate. Such an improvement was expected given that the overlapping between lactation, known for reducing performance (Castellini et al., 2006) and the resulting hormonal and energetic antagonism was virtually eliminated (Table 2).

The females in all experimental groups demonstrated similar body weight but at kindling, body weight falls were estimated at 7%, 15%, and 4.3% in the L, P-L and L groups, respectively.

The litter size at birth was higher ($p < 0.01$) in does of L group reflecting their better body condition. The litter size number were 9, 7 and 6.9 kits in does of L- P-L and P group, respectively. The corresponding values of average litter weight of does were 501 ± 9g, 402 ± 11g and 390.5 ± 13.6g. The higher milk yield during suckling period was in does of L group than milk yield of P-L group (2913 ± 55g *vs* 2200.7 ± 36g, $p < 0,05$). At weaning, litter size number became 8, 5, and 5.2 bunnies in does of L, P-L and P group, respectively.The corresponding values of average litter weight of does were 3720 ± 39g, 2200 ± 25g and 2040 ± 67g.The differences between doe milk yield during the suckling period may be due to differences in litter size between experimental groups. Concerning week of suckling period, the highest milk yield was during the 2nd week and the lowest milk yield was in the 1st week in all experimental groups (Table 2).

II.2. Serum proteins fractions and Lipids profile

The higher total protein, albumin and globulin concentrations ($p < 0.05$) were in does of L group (Table 3) while the lower concentrations were in E group. No significant difference was found in blood protein concentrations between simultaneously lactating and pregnant females and lactating females. A significant difference was recorded between the concentrations in all groups. Total lipids, triglycerides and total cholesterol concentrations in does of P group were ($p < 0.05$) higher than its concentrations in the rest of the experimental groups (Table 3).

II.4. Liver enzymes activities, Kidney function and Hormonal levels

Significant difference ($p < 0.05$) was found in enzyme activities pregnant doe, pregnant- lactating doe, lactating doe and empty doe (Table 3).

Uric acid and creatinine concentrations in does of P, P-L, and L groups were ($p < 0.05$) higher than in does of E group. Creatinine concentrations in simultaneously and pregnant (P-L group) doe were ($p < 0.05$) higher than its concentrations in lactating (L group) and pregnant (P group) doe. No significant difference was found in uric concentration between P, P-L and L groups.

Generally, uric and creatinine concentrations were still within the normal range (Table 3).

T3 and progesterone levels were ($p < 0.05$) higher in P and P-L groups, than in does of E and L group. A significant difference ($p < 0.05$) was found in T3 and progesterone and the rest of all experimental groups. Progesterone levels in does during pregnancy were ($p < 0.05$) higher than its concentrations during the suckling period.

Mean plasma glucose and insulin concentrations were ($p < 0.05$) higher in P and P-L groups. The lowest glucose and insulin values were ($p < 0.05$) in E group (Table 3).

III. DISCUSSION

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 may be due to the increased appetite of rabbits and consequently increased feed intake and good absorption in the intestinal tract (Okeke et al., 2011).Kids born of rabbits does of P and L groups weighed more and were of greater length than from does of P-L group (Rebollar et al., 2014). The difference between doe milk yields between experimental groups may be due to differences in litter size.

The increase in litter size and consequently litter weight of does at birthing may be due to the high number of ovulation and fertility in female rabbits of L group (Zerrouki et al, 2009; Theau-clement et *al.*,2013)).Glycemia can be a good reflection of the energetic balance of animals (Lamothe, 2006). 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 pregnant-lactating female rabbits (Fortun, 1994b) since the mammary gland is also a sensor for the synthesis of milk lipids.According to the obtained results, we see that the females started with an average glycemia (0.22 g/l). The latter increased during the second and third week of pregnancy (0.65 g/l) then diminished until parturition. 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 L group may be due to increase in feed intake and good absorption in the intestinal tract. 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 high lipemia ($p < 0.05$) can be explained by the energy needs that were more significant during pregnancy and lactation. Lipids produced more energy per molecule than glucose, but lipids oxidation required a higher consumption of oxygen than a complete oxidation of glucose. However, the quantity of ATP produced per a time unit from glucose was more significant than from lipids (Hocquette and al., 2000). 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 and al., 2005). Triglycerides were the constituents of lipoproteins. 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 does of P group may 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 triglycerides in Californian pregnant and lactating rabbit does.The hypolipidemia in P-L group may be due to suppression of hepatic lipogenesis (Sampath and Ntambi, 2005). The higher lipids fractions concentrations in does during pregnancy than its concentrations during suckling period may 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.

The decrease in ALT activity in does of all experimental groups may be due to a decrease in gluconeogenesis process in the liver. The increase in alkaline phosphatase in animals during suckling period may be due to an increase in milk biosynthesis (Salman, 2017).

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* 1.2-0.72 mg/dl (Okeke et al., 2011). 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 lactating-pregnant females.Generally, uric acid and creatinine concentrations were still within normal range. However, the increase in uric acid and creatinine concentrations in P and P-L groups may be due to the increase in feed intake and consequently the metabolic process. The lower uric acid and creatinine concentrations in E group than concentrations during pregnancy and suckling period may be increase these excretions from empty rabbits (Salman, 2017).

The T3 plasma levels were lower ($p<0.05$) in L group. Bracchia et al.,2005 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 (Bracchia et al., 2008).

Thyroid hormones, which are mainly implicated in the regulation of tissue growth and metabolism, are influenced by many factors, including nutrition this is supported by the finding of (Vanderpas, 2006) who 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) transport, sperm capacitation and oocyste maturation (Boiti, 2004).

The higher ($p < 0.05$) progesterone levels in P and P-L groups than its concentrations in E and L groups may be attributed to maintaining pregnancy.

CONCLUSION

As an original experiment is still in progress, it would be premature to draw any conclusion. However, some correlations are noteworthy. The lactating and pregnant females presented a strong mobilization of body fat, which can explain the energy needs that are more significant during pregnancy and lactation. It is also evident that excessive fatness of empty does should be controlled by reducing feed intake during the dry period.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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TABLES

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).

Parameters	Physiological status				P
	E(n=10)	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	ns
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)					
-at 1°week	-	-	435.1±96	631.4±136	*
-at 2°week	-	-	850.6±37	1316±156	**
-at3° week	-	-	935±67	966±155	ns
Average MY during suckling period	-	-	2200.7±36	2913.4±105	*
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	-	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.*

Table 3 - Effect of the physiological stage on metabolic profile and hormones in doe rabbits

Parameters	Physiological status			
	E(n=5)	P(n=5)	P-L(n=5)	L(n=5)
Total proteins (g/dl)	$3.60^c±0.5$	$4^b±0.90$	$5.70^a±1$	$6.10^a±0.1$
Albumin (g/dl)	$2.01^c±0.1$	$2.81^b±0.05$	$2.98^a±0.07$	$3.50^a±0.10$
Globulin (g/dl)	$1.22^c±0.02$	$1.69^b±0.01$	$1.88^a±0.06$	$2.80^a±0.04$
Total lipids (mg/dl)	$31^d±1$	$97^c±0.10$	$69^b±0.2$	$78.01^a±0.6$
Triglycerides (mg/dl)	$15.4^d±5.8$	$65.80^c±23$	$44.7^b±17.6$	$37.7^a±25.6$
Cholesterol (mg/dl)	$28.7^d±16.7$	$70^c±18.2$	$58.3^b±24.1$	$38.8^a±23.9$
ALT (IU/L)	$4.98^b±0.2$	$5.65^a±0.1$	$5.21^a±0.3$	$5.45^a±0.22$
AST (IU/L)	$18.34^a±0.4$	$22^a±1.3$	$21.4^a±1.14$	$20.35^a±4.3$
Alk-phosphatase (IU/L)	$12.8^b±0.85$	$40.50^a±8.9$	$39.8^a ±0.53$	$38.2^a±0.66$
Uric acid (mg/dl)	$7.9^d±1.4$	$21.2^c±3.8$	$34.8^b±3.4$	$24^a±3.3$
Creatinine (mg/dl)	$0.72^b±1.1$	$1.4^a±0.4$	$1.3^a±0.4$	$1.2^a±0.8$
T3 (ng/dl)	$22.4^c±0.53$	$31.6^a±0.6$	$44.3^b±0.93$	$29.2^a±0.6$
Progesterone (ng/ml)	$0.35^c±4.2$	$20^b ±0.2$	$18.98^b±1.5$	$10.7^a±0.15$
Leptin (ng/ml)	$1.5^a ±0.1$	$2.50^b±0.2$	$1.7^a±.3$	$2.02^b±0.9$
Insulin (µg/ml)	$22.2^a ±1.2$	$45.5^b±2$	$41.08^b±3.2$	$28^c±1.02$
Glucose (g/l)	$0.22^c ± 0.04$	$0.65^b ± 0.1$	$0.35^a ± 0.2$	$0.4^a ± 0.1$

a,b, c, d, Means in the same row within each physiological status having different superscripts differ significantly at P<0.05.