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Biochemical Profile of Local Rabbits *(Oryctolagus cuniculus*) During Successful Pregnancy Under Backyard Production System

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ABSTRACT

The aim of the current study was to establish reference plasma biochemical values for local rabbit (*Oryctolagus cuniculus*) during successful pregnancy under backyard production system in Peshawar Pakistan. Local rabbits (n=20) at day 13,18 and 28 of pregnancy and non-pregnancy were used. Experiments were executed using commercially available kits for the maternal plasma biochemical parameters including glucose, total protein, albumin, creatinine, Triglycerides, BUN, ALT and AST. During current study, the established value for glucose, total protein and albumin at day 13, 18 and 28 was 108±1.55, 115±2.7, 128.3±1.8 mg/dl; 56.4±1.69, 51.5±1.60, 46.0±1.84g/l; 39.2±1.1, 37.4±1.3, 34.1±1.05g/I whereas that of creatinine, triglyceride, BUN, ALT and AST at the corresponding days of pregnancy were 0.99±0.1, 0.81±0.2, 0.79±0.05mg/dl; 35.4±2.6, 57.9±2.8, 25.6±1.9mg/dl, 8.7±0.02, 15.7±0.13, 9.2±0.21mg/dl; 49.9±3.4, 47.8±1.07, 43.6±2.5 IU/l; 22.1±1.8, 23.9±1.6, 56.4±2.4 IU/l. Current findings demonstrated significant down-regulation of these biochemical indicators except BUN and AST during pregnancy period or at the placentation period and/or at the end of fetal growth of successful pregnancy in rabbits under backyard production system.

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HK designed and planned the project. AW, US and MKS executed the experimental work. MSQ statistically analyzed the result. SA wrote the article. MY and SK helped in writing the article.

Key words:
Local rabbit, biochemical profile, successful pregnancy, backyard production system.

INTRODUCTION

Improved rabbit production in the developing countries might play significant contribution in surmounting the meat requirement (Ajala and Balogun, 2004). The rabbit meat is nutritionally of high quality; low fat, sodium, and cholesterol levels and has a high protein level of about 20.8% (Biobaku and Oguntona, 1997). Additionally, the rabbit has high productive and reproductive aspects: prominent prospective for reproduction, fast growth rate, short pregnancy interval, abundant nutritional range, increased efficiency in converting forage to meat, increased prolificacy and comparatively reduced expenditure for production (Cheeke, 1986). Despites these distinctiveness, rabbit farming on large scale is not established in Pakistan hitherto; however these characteristic potentials herald the rabbit for the small holder subsistence-type integrated farming in the Khyber Pakhtunkhawa, Pakistan (Khan *et al.*, 2014). Therefore enhancement of the reproductive management of these rabbits would efficiently boost up its production under backyard production system.

Correct understanding of reproductive biology is fundamental in successful rabbit production. The pregnancy period is about 30-32 days. The placentation period is from day 13 to 18 day, during which extensive organogenesis occurs followed by fetal growth stage from day18 to 28. Furthermore, maternal tissues are associated with provision of energy for reproduction processes; growth and continued existence of developing fetus is strongly dependent upon availability of maternal nutrient such as Glucose, Lipids and Proteins etc in the course of pregnancy (Vallet *et al*., 2002). These nutrients together with other biochemical constituents are significant indicators of physiological state and the reproductive performance (Perveen and Usmani, 1993; Prabha *et al.*, 2000). Furthermore, the investigation of enzymatic activity *i.e.*, alanine transaminase (ALT) and aspartate transaminase (AST) is indispensable in rabbits rearing for assessment of the various disorders of enegery metabolism or damages encountered in the visceral organs including liver and kidney (Krupczynski and Chudoba-Drozdowska, 2002; Darul and Kruczynska, 2005; Jurcik *et al.*, 2007; Melillo, 2007; Jenkins, 2008). Therefore, utilization of maternal blood biochemical profile is a relentless requisite in the assessment of the well-being, health and nutritional status of mammalian species (Gupta *et al.*, 2007).

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Several researchers have investigated reproductive characteristics especially in relation to biochemical profile of the maternal plasma or serum in various rabbit breeds during entire pregnancy period in different regions of the world (Al-Eissa *et al.*, 2012; Khan *et al.*, 2011; Ozung *et al.*, 2011; Hafez and Tsutsumi, 2005; Balogh and Sotonyi, 2003) under diverse environmental condition. However, no data is available on the expression of these profiles in maternal plasma during successful pregnancy in the local rabbits under the small holder subsistence type integrated farming system in the province thus far. In addition, controlled feeding during pregnancy is essential to evade excessive fattening and high mortalities throughout pregnancy (Rommers *et al.*, 2001). Consequently, the current pilot study was investigated to establish reference normal range of biochemical constituents as a gauge of the metabolic requirements at different stages of successful pregnancy in local rabbit under small scale, backyard production system in Peshawar, Pakistan.

MATERIALS AND METHODS

The present research project was conducted in accordance with guidelines of the ethical committee of Faculty of Animal Husbandry and Veterinary Science, The University of Agriculture, Peshawar. A total of 20 female nondescript local rabbits were purchased from rabbit breeders/farmers having the breeding or animal facility that were bred under normal condition with known pregnancy days and non-pregnancy. The pregnant local rabbits were purchased at day 13, 18 and 28. These days play a vital role in the reproductive biology of rabbits since from day 13 to day 18 is the placentation period whereas from day18 to 28 is the fetal growth stage during successful pregnancy. The mating day was designated as day 0 of pregnancy. The blood samples were collected aseptically from the auricular ear vein on day 13, 18 and 28 of pregnancy and non-pregnant rabbits. For plasma 3ml of blood was collected in EDTA containing tubes with sterilized 3ml disposable syringe followed by centrifugation (Centrifuge 80-2, China) at 3000 rpm for 10 min to collect plasma for analyses. It was stored at -18 to -20 ^{0}C until it was used for biochemical analysis (Tabatabaei, 2011). Measurement of concentration of glucose, total protein, albumin, blood urea nitrogen (BUN), triglyceride, creatinine, alanine transaminase (ALT) and aspartate transaminase (AST) in maternal plasma were conducted using commercially available kits (Reactivos GPL, Chemelex, S.A. Barcelona, Spain) according to the manufacturer's instruction.

Statistical analysis

Statistically the mean values (±SE) for concentrations of various biochemical compositions of maternal plasma were calculated. Expression of biochemical profile during pregnancy or non pregnancy was presented as Mean±SE using SPSS (Statistical Package for the Social Sciences). One way ANOVA (analysis of variance) was used to indicate the level of variation in values of various biochemical profiles in maternal plasma during different stages of pregnancy. Duncan's multiple range tests was applied to show the level of significance between means. Statistical significance was set at $P<0.05$.

RESULTS

During current study, the established value for glucose, total protein and albumin at day13,18 and 28 was 108.7±1.55, 115.6±2.7, 128.3±1.8 mg/dl; 56.4±1.69, 51.5±1.60, 46.0±1.84g/l; 39.2±1.1, 37.4±1.3, 34.1±1.05 g/l whereas that of creatinine, triglyceride, BUN, ALT and AST were 0.99±0.1, 0.81±0.2, 0.79±0.05mg/dl; 35.4±2.6, 57.9±2.8, 25.6±1.9 mg/dl; 8.7±0.02, 15.7±0.13, 9.2±0.21 mg/dl; 9.9±3.4, 47.8±1.07, 43.6±2.5 IU/l; 22.1±1.8, 23.9±1.6, 56.4±2.4IU/l. Corresponding days for non-pregnant rabbit showed higher values for these biochemical variables (Table I). Furthermore, glucose concentration was significantly increasing from placentation period to fetal growth stage (Table I). Also total protein and albumin concentration in maternal plasma was found significantly higher at early stage of pregnancy whereas significant decrease has been recorded with each succeeding stage of pregnancy (Table I). Creatinine (Creat) and triglyceride (TG) concentration in maternal plasma of rabbits was significantly decreased with progression of pregnancy than non-pregnant stage (Table I). Moreover, significant increase was observed during placentation period (day18) whereas a significant decrease was recorded during the fetal growth stage (Table I). Significant reduction was recorded in the concentration of urea in the maternal plasma at early placentation (day 13) and at end of fetal growth stage (day 28) in comparison with non-pregnancy, on the other hand,a significant increase has been observed at the placentation period (day18) (Table I). In the current study, ALT concentration in maternal plasma was found significantly lower during pregnancy when compared with non-pregnancy, whereas AST concentration in maternal plasma was found significantly higher from the period of extensive organogenesis to fetal growth stage of successful pregnancy.

Table I.- Biochemical profile of successful pregnancy during placentation period and fetal growth period of local rabbits under backyard production system (n=20).

Biochemical indicator	Non-pregnant Day 0 (n=5)	Stages of pregnancy		
		Placentation period		Fetal growth period
		Day 13 (n=5)	Day 18 (n=5)	Day 28 (n=5)
Glucose (mg/dl)	134.5±1.47[a]	108.7±1.55[b]	115.6±2.7[c]	128.3±1.8[d]
Total protein (g/l)	58.1±2.05[a]	56.4±1.69[a]	51.5±1.60[b]	46.0±1.84[c]
Albumin(g/l)	41.3±0.9[a]	39.2±1.1[b]	37.4±1.3[b]	34.1±1.05[c]
Creatinine (mg/dl)	1.4±0.3[a]	0.99±0.1[b]	0.81±0.2[c]	0.79±0.05[c]
Triglyceride (mg/dl)	65.1±4.1[a]	35.4±2.6[c]	57.9±2.8[b]	25.6±1.9[d]
BUN(mg/dl)	13.2±0.2[b]	8.7±0.02[d]	15.7±0.13[a]	9.2±0.21[c]
ALT(IU/l)	61.2±2.6[a]	49.9±3.4[b]	47.8±1.07[b]	43.6±2.5[b]
AST(IU/l)	23.6±1.3[b]	22.1±1.8[c]	23.9±1.6[b]	56.4±2.4[a]

Different superscript a-d in the row indicated level of significance among different stages of pregnancy. n, the number of rabbits used at each point of pregnancy; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Level of significance was $p<0.05$.

DISCUSSION

To our knowledge, this is the first study to investigate differential expression of biochemical indicator in different physiological stages of successful pregnancy of local rabbits under backyard production system. It has been demonstrated that normal levels of biochemical constituents are not only essential in sustaining the functional integrity of the reproductive system but also play a significant role in diagnosis of various productive and reproductive disorders in mammalian species (Meenakshi *et al.,* 2011). The availability of maternal nutrient such as glucose, lipid and amino acid through uterus is fundamental for growth and survivability of developing fetuses (Trujillo-Ortega *et al.,* 2006; Vallet *et al.,* 2002). In the current study, distinct variation in glucose, total protein and other biochemical indicator were observed in different physiological stages of the successful pregnancy that might indicate the functional adjustment of local rabbits to succeeding developmental phase from early placentation (day 13) to end of the fetal growth (day 28).

Glucose being indispensable source of energy is crucial for reproductive activities (Radostits *et al.,* 2000). In current study, significantly lowered Glucose concentration has been recorded in the maternal plasma of pregnant rabbits that were consistent with those recently reported in News Zealand white rabbit, Japanese white rabbits and angora rabbits (Haneda *et al.,* 2010; Mizoguchi *et al.,*2010; Khan *et al.,* 2011). Their findings also demonstrated differential expression in maternal glucose concentration at different stages of pregnancy. It could be figured out from these report including our current finding that lowered glucose concentration in maternal plasma of pregnant rabbit might be associated with provision of glucose availability to developing fetus according to nutritional requisite during pregnancy (Wells *et al.,* 1999).

Total protein has been generally utilized for assessment of nutritional status of an animal indicating food ingestion and metabolism. The lower value of the total protein in maternal plasma recorded in current study during successful pregnancy was in agreement with the findings obtained by Brzostowski *et al*. (1996) who reported lowered protein level during pregnancy. Also, the results obtained in current study were in accordance with findings of Mizugochi *et al.* (2010), Cetin *et al.* (2009), Ozegbe (2005), Wells *et al.* (1999) in New Zealand white rabbit, Japanese white rabbits and Angora rabbits. The possible reason for the lowered total protein concentration in maternal plasma during the entire pregnancy period in the current study might be associated with increased growth of the developing fetus, particularly the consumption of amino acids from the maternal distribution for protein synthesis in the fetal muscles (Antunovic *et al*., 2002; Jainudeen and Hafez, 1994). Furthermore, current finding related with albumin concentration were in agreement with Mizoguchi *et al*, (2010) and Wells *et al.* (1999) in New Zealand white rabbits and Cetin *et al.* (2009) in angora rabbits. It has been suggested that the decrease in the albumin contents might be attributed to increase blood volume that occurred due to haemodilution during successful pregnancy.

Our results regarding creatinine concentration in maternal plasma corresponds with the findings of Mizoguchi *et al.* (2010) and Wells *et al.* (1999) who studied New Zealand white rabbits during successful

pregnancy. Furthermore, previous research conducted in several mammalian species including pregnant rabbits (wells *et al*., 1999), human (Stock and Metcalfe, 1994) and rat (Baylis, 1980) has demonstrated that the lowered concentration of Creatinine in the maternal plasma has been associated with increased glomerular filtration rate during pregnancy. Taken together, including our current study, it seems reasonable that the decreased creatinine in the maternal plasma in these local rabbits may reflect a comparable functional phenomenon.

The results of the current study concerning concentration of the triglyceride in pregnant does were in line with recent finding of the Abu El-Ella *et al*. (2014), Giuseppe *et al.* (2009), Cetin *et al.* (2009) and Wells *et al.* (1999), who demonstrated significant lower value of plasma triglyceride in pregnant does as compared to non-pregnant animals during the entire pregnancy. Additionally, it has been demonstrated that triglycerides are the storage lipids of mammals in the plasma and the body can use it for fuel under physiological adjustments. The utility of the plasma lipid by the dam has been demonstrated during the second half of pregnancy; the period of extensive organogenesis in rabbits for which enormous amount of energy is required (Ozegbe, 2005). Moreover, the significant decline in triglycerides concentration in maternal plasma after the period of extensive organogenesis (day 18) may reflect the fast deregulation of the rabbit's energy metabolism at the fetal growth stage during pregnancy. BUN concentration in the maternal plasma recorded in current study were consistent with the study of Mizoguchi *et al.* (2010), Haneda *et al.* (2010) and Wells *et al.* (1999). These researchers elucidated the reduced value of maternal plasma urea concentration in different breeds of rabbits during successful pregnancy in diverse environmental condition. Conversely in our study, the maximum concentration of the urea in maternal plasma has been observed at day 18 which is the peak period not only for extensive organogenesis of the developing fetus but also completion of the rabbit placental maturation. It seems reasonable that increased concentration of maternal urea of pregnant rabbit might be ascribed to high demand of energy during this period. It has been demonstrated that increase in maternal BUN concentration during second half of pregnancy might be associated with the prominent provisions of energy (Piccione *et al*., 2009). Also, increased protein metabolism during different reproductive stages of pregnancy might be other reason for increased maternal BUN concentration (Gurgoze *et al*., 2009). Both ALT and AST are generally used as gauge for the assessment of liver function as well as well-being of the mammalian species during pregnancy (Khatun *et al*., 2011). Several researchers have regarded AST and ALT in the blood plasma or serum as a useful device to diagnose diseases of organs and tissues (Stec *et al*., 2006; Ramin *et al*., 2005). Furthermore the movement of amino transferases in blood is essential as it works as a catalyst in relation with the metabolism of amino acids and carbohydrates. Significant decrease that has been observed for ALT concentration in maternal plasma during the period of organogenesis and fetal growth of the pregnancy in the current study is in agreement with the finding of Mahawar *et al.* (2004) and Pouroucholtamane *et al.* (2005) in different mammalian species. Also, current results regarding AST concentration of maternal plasma is in agreement with the findings of Wells *et al.* (1999) in the New Zealand white pregnant rabbits.

In conclusion, the current study established the reference values for selected biochemical constituents in the maternal plasma in the local rabbits for the small holder subsistence-type integrated farming under backyard production. Additionally the current reference values might be relevant for prenatal detection of alteration in metabolism of these nutrients, thus proper remedial steps could be taken to surmount the metabolic disturbances during pregnancy to increase its production under backyard production system.

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Conflict of interest statement

All authors have no conflict of interest with any one about this manuscript.

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