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Reproductive and Haematological Responses of Breeder Rabbits Offered Two Dietary Regimes

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Abstract: Reproductive and haematologic responses of breeder rabbits offered two dietary regimes were studied. Twenty yearling does were used at five stages of pregnancy in a 2×5 factorial experiment involving the use of two types of diets (forage mash and mash alone). Serum chemistry values were determined at selected gestation periods in the rabbits. Total protein, albumin, glucose, aspartate aminotransferase and alanine aminotransaminase values decreased significantly with advancing gestations and recovered to about the initial (pre-pregnant) levels by 4-5 days post parturient. However, alkaline phospatase levels increased as gestation advanced but increased to about the initial pre-pregnant levels at about the same period after gestation. There was no statistical difference ($p>0.05$) between the dietary treatments. Similarly, differences in feed intake, weight gain, conception and kindling rates and number of rabbits weaned were not statistically different between the dietary regimes. Results showed that irrespective of the types of diet offered in the experiment, values of the serum components examined decreased systematically during pregnancy. The rabbit however tended to recover to values approaching pre-pregnant levels 4-5 days after kindling. The studies showed that the protein level (12.58%) in the commercial (poultry) grower ration used was inadequate to support optimum reproduction in rabbits and as a result farmers should supplement such diets with more protein to achieve the recommended 18% crude protein requirement. Similarly, forage use in the nutrition of rabbits should contribute fibre levels not exceeding 17% of the ration.

Key words: Reproduction, haematology, rabbits, dietary regimes

INTRODUCTION

Exposure to various environmental conditions including diets influence animal performance and physical state. The haematological status of animals have been used as an index of such physiological conditions (Le Resche *et al*., 1974). Results obtained from such assessment could contribute to improvements in management procedures. Total serum protein levels for instance may reflect the nutritive state of an animal. Higher serum protein and albumin levels were observed in bears foraging on a relatively abundant source of food (Siperek, 1979). In starving animals the carbohydrate and protein contents in blood have been observed to decline (Florey, 1966).

Haematology and serum chemistry are important diagnostic tools in veterinary medicine. Clinicobiochemical studies in pregnancy toxaemia in sheep indicated a significant fall in blood glucose, total serum protein and albumin, while significant increases were observed in the levels of ketone bodies, total volatile fatty, acids, blood urea nitrogen, creatinine and cholesterol (Singh *et al*., 1992).

In pregnant animals, maternal blood supplies the foetus with all its nutrients. Studies show that the quantity of blood reaching the foetus determines its rate of growth and development. High levels of specific blood nutrients stands to withstand the stresses of pregnancy, parturition, lactation and brooding better than those with lower levels.

Studies reveal that additional demands for systemic nutritive substances are imposed on adult females during pregnancy and lactation (Rosso and Crammy, 1979). However, the information on the relationship between such demands and specific reproductive events in some farm animals have not been widely investigated.

Information on biochemical and haematological parameters can be valuable indicators of the physiological status of an animal in any environment. Such data in respect of the breeder rabbit under tropical humid conditions seem to be limited in scientific literature.

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This study was therefore undertaken to investigate levels of certain blood biochemical parameters as well as haematological characteristics of breeder rabbits at selected gestation and post parturient periods under two dietary regimes.

MATERIALS AND METHODS

Twenty yearling does of New Zealand white rabbits were used where animals were randomly subdivided into five groups of two does per group. They were housed in conventional rabbit hutches made of wire mesh and iron frame. Each compartment was equipped with aluminium drinkers and feeders. The hutches were housed in a dwarf-walled house with the top half covered with wire mesh. The animals were pre-conditioned for two weeks during which they were fed a diet of commercial poultry (growers) mash and forages (*centrosema pubescens*, *peuraria phasioloides* and *panicum maximum*). Medication with 5g amprolium in 4 litres of water was used during the period to control an observed incident of coccidiosis.

A 2×5 factorial (completely randomised) experimental design was used. The two factors were types of diet offered and stages of pregnancy. The two diets were mash alone and forage/mash combination, while the pregnancy stages were; pre-pregnancy, early pregnancy (8-9 days), late pregnancy (25-26 days) and post parturient (4-5 days).

Mating of the does started immediately after the preconditioning period and lasted one week. All does except the pre-pregnant group were bred. Does ready to submit to mating were determined by observation of vital oestrus signs, viz; vascularization and swelling of the vulva, exposition of the rear quarters, rubbing of the chin on feed/water trough, aggressive restlessness and mounting pen mates. However, those not showing signs of heat were forced mated (Berepubo, 1995), towards the end of the one week monitoring period.

The mating ratio was one breeding buck: Four which were normally introduced into the back's pen. Mating was believed to occur when the buck fell sidewards at the end of coitus. Each doe was mated at least twice within two days.

Pregnancy diagnosis was done by the abdominal palpation procedure as described by Berepubo (1995) in combination with close observation on the condition of the mammary organ and the loss of fur around the abdominal region.

The experimental diets comprised 150 g forage combined with 100 g poultry grower mash/animal/day for the forage/mash group and 100 g grower mash per doe per day solely in all mash group. The forages were usually cut fresh in the morning, tied close to the feeding trough (containing mash) in the cages. Both forages and mash were supplied simultaneously.

Table 1: Chemical composition of experimental diets (Forages and Mash) percentage

Nutrient	Centrosema pubescens	Pueraria phasioloides	Panicum maximum	Mean	Commercial grower mash
Dry matter	42.04	40.50	38.10	40.30	93.05
Crude fat	0.80	0.60	1.00	0.80	5.80
Crude protein	29.35	30.40	8.50	22.75	12.58
Ash	6.30	7.70	9.00	7.67	6.00
Crude fibre	25.40	27.80	32.40	28.53	0.13

Samples of the experimental diets were collected into well-labelled cellophane bags and transferred to the laboratory for analysis. The diets were chemically analysed for dry matter, crude fat, crude protein, ash and crude fibre using the method of AOAC (1984) Table 1.

Body weight and weekly feed intake was assessed and reproductive traits (conception rate, kindling and weaning ability) were recorded.

Biochemical and haematological assessment: Five millilitres of blood was obtained from each animal at the different stages of pregnancy by venipuncture. Samples were collected into clean glass tubes and labelled. Sera were subsequently harvested and stored at 4°C until used. All blood samples were analysed within 24 h. Glucose was determined by method of Biotech Laboratories (1985). The protein fraction, (total protein, albumin and bilirubin) and the enzymes, (aspartate Aminotrsnsferase (AST), Alanine Aminotrasferase (ALT) and Alkaline Phospatase (ALP) were analysed by the methods of Kachmar and Grant (1976), Kachmar and Moss (1976), respectively.

Resultant data were analysed by analysis of variance procedure of Gil (1978). Significant difference between mean values were examined by Duncan's Multiple range test (Steel and Torrie, 1981).

RESULTS

Body weight changes: The mean weight gain and feed intake are presented in Table 2. In both treatments, late pregnancy does had higher body weight gain (numerically), (11.50±1.27 g forage/mash versus 11.25±2.43 g mash) than the other group of does. Similarly, daily weight gain in pre-pregnant, early, middle and post parturient phases did not differ significantly.

Feed intake: Differences in daily feed intake were not significant ($p>0.05$) in all the treatments. However, the highest numerical values were observed in both the

Table 2: Effects of mash and forage/mash diets on some performance characteristics in rabbits at selected gestation and post parturient periods

Parameters	Dietary treatment	Stages of pregnancy: Pre preg.	Early preg.	Middle preg.	Late	Post preg. parturient
Initial weight(kg)	M	1.26±0.03	1.25±0.02	1.23±0.00	1.22±0.02	1.22±0.01
	MF	1.24±0.02	1.21±0.01	1.23±0.01	1.21±0.01	1.20±0.02
Final weight(kg)	M	1.82±0.09	1.95±0.28	1.60±0.13	2.10±0.09	1.50±0.23
	MF	1.62±0.15	1.78±0.11	1.65±0.12	2.05±0.28	1.67±0.10
Total weightGain (kg)	M	0.56±0.03	0.53±0.00	0.54±0.01	0.68±0.15	0.35±0.18
	MF	8.00±1.23	9.56±0.35	10.58±1.35	11.50±1.27	7.50±1.73
Daily feed intake (g)	M	69.00±1.60	72.50±1.90	71.50±0.90	71.50±0.40	69.00±1.60
	MF	68.00±1.90	72.00±2.10	72.50±2.60	68.50±1.40	68.50±1.40

M = Mash, MF = Mash and Forage

Table 3: Effects of mash and forage/mash diets on some reproductive characteristics in rabbits at selected gestation and post parturient periods

Parameters	Dietary treatment	Stages of pregnancy: Early preg.	Middle preg.	Late preg.	Post parturient
Conception rate	M	0.50±0.10	0.50±0.10	1.00±0.40	1.00±0.40
	MF	0.50±0.10	0.50±0.10	1.00±0.40	1.00±0.40
Kindling rate	M	1.95±0.28	1.60±0.13	2.10±0.09	1.50±0.23
	MF	1.78±0.11	1.65±0.12	2.05±0.28	1.67±0.10
Weaning rate	M	0.53±0.00	0.54±0.01	0.68±0.15	0.35±0.18
	MF	9.56±0.35	10.58±1.35	11.50±1.27	7.50±1.73

M = Mash, MF = Mash and Forage

Table 4: Values of some blood biochemical constituents in different stages of reproduction in rabbits

Parameters	Stages of pregnancy: Pre preg.	Early preg.	Middle preg.	Late preg.	Post parturient
Glucose (mg dL^{-1})	$94.60±8.13^{a}$	$85.20±1.27^{bc}$	$85.20±1.27^{bd}$	$81.17±5.30^{c}$	$86.20±0.27^{ab}$
Total protein(mg dL^{-1})	$07.10±1.12^{a}$	$06.10±0.12+^{ab}$	$05.20±1.78^{b}$	$04.60±1.38^{c}$	$06.90±0.92^{a}$
Albumin (g dL^{-1})	$03.10±0.56^{a+}$	$02.60±0.24^{b}$	$02.30±0.24^{b}$	$01.80±0.56^{c}$	$02.29±0.36^{a}$
Total bilirubin(mg dL^{-1})	$01.73±0.42^{a}$	$01.29±0.22^{bc}$	$01.19±0.32^{c+}$	$01.40±0.11^{b}$	$01.75±0.35^{a}$
Conjugated bilirubin (mg dL^{-1})	$00.82±0.26^{a}$	$00.474±0.09^{cd}$	$00.35±0.21^{d}$	$00.53±0.03^{c}$	$00.64±0.08^{b}$
AST (IU L^{-1})	$15.00±3.20^{a}$	$13.00±1.24^{a}$	$11.00±0.76^{ab}$	$09.20±1.80^{b}$	$10.60±0.52^{b}$
ALT (IU L^{-1})	$07.60±0.88^{a}$	$06.40±0.88^{b}$	$04.50±1.02^{b}$	$04.10±1.42^{b}$	$05.00±0.52^{b}$
ALP (IU L^{-1})	$23.00±1.60^{c}$	$24.00±0.06^{b}$	$26.00±1.40^{b}$	$30.00±5.40^{a}$	$20.00±4.60^{c}$

a,b,c-means with different superscript on the same row are significantly different. AST = Aspartate amino transferase, ALT = Alanine amino transferase, ALP = Alkaline phosphatase

middle pregnant (72.50±2.60 g) and early pregnant groups (72.50±1.90 g) for mash/forage and mash feeding systems respectively. In both dietary groups numerical values for daily feed intake was lower at the post-parturient stage (68.00±1.40 forage/mash versus 69.00±1.60 g mash).

Reproductive performance: Statistical analyses indicate that there were no significant differences ($p>0.05$) between animals fed mash diets and those fed forage/mash combination (Table 3).

The numerical difference in conceptions rate seemed large for the late pregnancy groups than other groups in both dietary treatments.

For kindling rate high numbers ($p>0.05$) were observed at post parturient (5.00±2.80) stages for mash fed and forage/mash fed rabbits respectively.

Late pregnancy (3.50±2.30) rabbits on the forage/mash weaned more kittens than others dams given similar treatment. Similarly, in the mash fed rabbits the middle pregnancy (2.00±1.50) dams weaned more kittens.

Serum chemistry: Data on serum chemistry at different stages of gestation are presented in Table 4. The values differed significantly ($p<0.05$) between the reproductive groups. The data shows that as gestation advanced, serum glucose, total protein, albumin, bilirubin, aspartate aminotransferase and alanine minotransferase levels decreased. However, alkaline phosphate concentrations increased with advancing gestation.

The decrease in blood glucose value between different gestation period were statistically significant ($p<0.05$). blood glucose concentrations at pre-pregnancy (94.60±1.27 mg dL^{-1}), were significantly ($p<0.05$) higher than those at early pregnancy (85.20±1.27 mg dL^{-1}), middle pregnancy (85.20±1.27 mg dL^{-1}) and late pregnancy (81.17±30 mg dL^{-1}). The post parturient value of 86.20±0.027 mg dL^{-1} was significantly ($p<0.05$) higher than at late pregnancy.

Total Protein (TP) levels in pre-pregnant animals (7.10±1.12 g dL^{-1}) and at the post parturient (6.90±0.02 g dL^{-1}) physiological phase were significantly higher ($p<0.05$) that at late (4.60 ± 1.39 g dL^{-1}) and middle (5.20 ± 0.78 g dL^{-1}) pregnancy but not significantly ($p>0.05$) different from the level at the early pregnancy phase (6.10 ± 0.12 g dL^{-1}).

Similarly, albumin level in at pre-pregnancy (3.10 ± 0.56 g dL^{-1}) was significantly higher (p<0,.05) than the late (1.80 ± 0.56 g dL^{-1}) and middle (2.30 ± 0.34 g dL^{-1}) pregnancy, but similar at the post-parturient (2.90 ± 0.36 g dL^{-1}) and in early pregnancy (2.60 ± 0.06 g dL^{-1}) respectively. However, albumin concentration at middle pregnancy was significantly ($p<0.05$) higher than that of late pregnant phase.

Total bilirubin concentration in non-pregnant (1.73 ± 0.42 mg dL^{-1}) and post parturient (1.75 ± 0.35 mg dL^{-1}) animals were higher ($p<0.05$) than at middle (1.19 ± 0.32 mg dL^{-1}), late (1.40 ± 0.11 mg dL^{-1}) and early (1.29 ± 0.27 mg dL^{-1}), pregnancy stages. Levels of serum total bilirubin at early and middle pregnancy were lower ($p<0.05$) that at late pregnancy.

Conjugated bilirubin value in pre-pregnant (0.82 ± 0.26 mg dL^{-1}) animals were higher ($p<0.05$) than in late (0.53 ± 0.03 mg dL^{-1}), early (0.47 ± 0.09 mg dL^{-1}) and middle pregnancy (0.35 ± 0.21mg dL^{-1}) as well as post-parturient (0.64 ± 0.09 mg dL^{-1}). Conjugated bilirubin at the middle pregnancy significantly differed ($p<0.05$) from late pregnancy.

Aspartate aminotransferase concentrations in the serum of pre-pregnant and early pregnant animals did not differ ($p>0.05$). These values were however, higher than ($p>0.05$) from the value at early pregnancy.

Alanine aminotransferase concentrations in the serum of pre-pregnant animals was significantly higher ($p<0.05$) than those of pregnant and post-pregnant animals. The concentration in pregnant and post-parturient animals did not differ ($p>0.05$).

The serum levels of Alkaline Phospates (ALP), increased numerically with the advancement of pregnancy. A significantly high ($p<0.05$) level (30.00 ± 5.40 lu L^{-1}) was observed during late pregnancy which differed from the physiological stages.

DISCUSSION

The average weight gain in all the treatments were not significant ($p>0.05$). The weight gains for pre-pregnant rabbits in this study agreed with those reported by Ngodigha *et al.* (1994) but were lower than values reported by Cheeke and Patton (1987) and Deshmukh *et al.* (1990). The differences with the observation of the later workers may be due to the environmental differences in the locations of the studies (temperate versus tropical). Weight gains in pregnant and lactating rabbits were lower than the values reported by Aduku (1993). This may be due to the low protein content of the mash diet *(12.58%) in this study against the recommended requirements of (18%). The higher level of crude protein of (22.75 and 12.5%) in the forage/mash treatment did not however, bring about a corresponding increase in weight gain. It is possible that the high fibre content of forages which is associated with decreased dry matter intake (Labas, 1984) was responsible for this observation. Cheeke (1983), Davidson and Spreadbury (1975), Crowder and Chedda (1982); Harris *et al.* (1983) observed that feed intake in rabbits decreased when dietary crude fibre level was higher than 17%. High crude fibre content probably decrease palatability of the diet.

It is also possible that there are substances in the experimental forages which bind and therefore reduce the availability of the nutrient. Chlorogenic acid, oxalates, tannin, cynanide, mimosine, lucaena and haemaglutinins have been reported in forage species (Cannor *et al.*, 1980; Piepoint, 1983; Harris *et al.*, 1981; Cheeke and Shull, 1985).

The conception, kindling and weaning rates of rabbits in the present study were relatively low compared to reports by Berepubo (1993). This may again be due to the low protein content of the diet as well as poor availability of nutrients in forages to the animals. Sod-Moriah (1971) reported that under-nutrition undoubtedly reduces fertility in female animals. According to this worker, this seems have a greater impact on pregnancy and lactation than on ovulatory cycles, although cycles may become irregular. The time of implantation is generally normal (Sod-Moriah and Bedrock, 1973), but Fewer Corpora Lutea are reported (Pasley and Mikinney, 1973). Uterine weights and response to exogenous oestrogen are also reduced (Bolt *et al.*, 1996). Maternal blood supply to the placenta and foetus becomes poorer (Widdowson, 1981).

Information on serum chemistry of rabbits at pregnancy and post-parturient stages are relatively scanty in published sources.

Mammals however, appear to have similar biochemical patterns. Man's biochemical patterns for instance are relatively similar to that of rabbits, such that rabbits are often used in animal model studies involving variables in man (Bortolotti *et al.*, 1989).

In general, serum values for non-pregnant animals in the present study similar to the range of reference value for rabbits (Mitruka and Rawnsley, 1981; Lepitzki and Woolf, 1991; Jacobson *et al.*, 1978a). The range of values for enzymes were however lower (especially for AST and ALT) than reports from other studies. Mitruka and Rawnsley (1981), reported thatserum enzyme levels varied widely in the experimental rabbits. Lepitzki and Woolf (1991) also indicated differences due to the assay methods applied.

There were decreases in the serum levels of glucose, total protein, albumin and bilirubin during pregnancy particularly in the late phase. This is due to the additional nutritional demands by the conceptus, especially at the period of maximum foetal growth. The fetuses may revive certain nutrients in excess of their requirements to obviate the possibility of shortages (Rosso and Crammy, 1979). The dam's intake is distributed to enable more than ample supply to te conceptus. This is made possible because nutrients are distributed among tissues according to metabolic needs. Since the conceptus would have a higher metabolic rate than the maternal tissues, it would receive more nutrients per unit of body than the mother (Hammond, 1994). A similar observation was made in cows by Setia *et al.* (1992), sheep and goats (Rwuaan *et al.* 1993) and Man (Ermalinda and Fiereck, 1982). Hugget and Morrision (1955) observed that during the last third of gestation in rabbits, the decrease in Placental glycogen was accompanied by a reciprocal increase in the glycogen of the foetal liver.

The decrease in the glucose from early pregnancy (day 8-9) was probably due to the implantation of the blastocysts which occurs about this time, marking the beginning of glucose extraction from the dam by the conceptus. Austin (1961) and Braden (1960) studied the biochemical fluid content of rabbit blastocysts just before and during implantation (6, 7 and 8 days from coitus). The fluid was found to contain very little glucose on day 6, a higher level on day 7 and amounts approaching the serum concentration on day 8. The Workers reported that the glucose passed to the blastoctysts from the maternal blood stream. However, the higher values of blood glucose concentration during the post-parturient period (as against pregnancy values) may be due to improved glucose turnover rate and increased insulin responsiveness during lactation. Blood glucose turnover rate is enhanced during lactation in rabbits (Pere *et al*., 1992, Hugget and Morrision, 1955). Similar observation was reported in cows (Bickerstaffe *et al*., 1974), goats (Buckley *et al*., 1982) and sheep (Bergman, 1963; Bergman and Hogue, 1967; Wilson *et al*., 1983).

Since the post-parturient blood glucose levels were not significantly lower as compared to pre-parturient values, there is the probability that the animals could be recovering from the stress of glucose extraction during pregnancy at the period.

Similar inferences may be extended to the decrease trend observed in the total protein, albumin and mbilirubin levels. Ermalinda and Fiereck (1982) reported that the decrease in both total protein and albumin levels with gestational age may be as low as 1/10 of normal serum level during the 2nd trimester and 1/20 near term in man. Apart from foetal extraction, the fall in protein fractions reflect an extra requirement by the dam for blood volume expansion, growth of uterus and breasts, placenta, amniotic fluid, other maternal tissues and organs associated with pregnancy and lactation. The rise in the level of Alkaline Phosphates (ALP) during pregnancy is not well understood. However, investigators (National Academy of Sciences, 1977) have suggested that other issues may be secreting ALP in addition to the 70% unused ALP secreted by the placenta. This may account for the increase in the concentration of ALP during pregnancy.

The variations in the levels of AST during pregnancy and post parturient periods in the rabbits studied is not very clear. However, Kachmar and Moss, (1976) associated elevated serum levels of AST to disease processes affecting liver cells. Other diseases that may cause the elevation include regional enteritis and intra-abdominal bacterial infection. Observations in the present study may be due to the effects of pregnancy.

The later inference may be extended to the variations observed in the levels of ALT, between the pre-pregnant and pregnant stages. ALT levels from early pregnancy to post parturient periods did not differ ($p>0.05$) agreeing with National Academy of Sciences (1977) that the ALT levels remains relatively constant throughout pregnancy.

CONCLUSION

It was concluded from this study that a protein level (12.58%) in the commercial (poultry) grower ration used in the present study is inadequate to support optimum reproduction in rabbits. It is therefore recommended that farmers should supplement such diets with more protein to achieve the recommended 18% crude protein requirement.

Similarly, forages used in the nutrition of rabbits should contribute crude fiber levels not exceeding 17% of the diet.

Serum biochemical observations suggest that rabbits tend to recover from pregnancy stress in about 4-5 days after kindling.

However, further studies on the serum biochemical constituents of rabbits at various stages of pregnancy and lactation are required.

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