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Full Length Research Paper

Assessment of serum biochemistry in West African Dwarf (WAD) does administered varying levels of medroxyprogesterone acetate (MPA), an estrus synchronizing drug

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This study was carried out to assess the health status (serum biochemistry) of West African Dwarf (WAD) does administered varying levels of medroxyprogesterone acetate (MPA), an oestrus synchronizing drug. The experiment was laid out in a completely randomized design. Result of the experiment showed that all the does were in good health status before, during and after the drug administration. Blood serum electrolytes analyses showed that all parameters assessed were within the normal range of a healthy goat. Sodium (Na^+) mEq/L and potassium (K^+) mEq/L were not significantly ($P > 0.05$) affected, by the drug administered. Urea (mg/dl) and creatinine (mg/dl) were significantly ($P < 0.05$) influenced by the drugs administered. Serum enzymes activities in WAD does showed that alanine transaminase (ALT) (m/L) and aspartate transaminase (AST) (m/L) were not significantly affected ($P > 0.05$) by administering MPA drug but alkaline phosphatase (ALP) (mg/dl) was significantly ($P < 0.05$) influenced before and during the drug administration. Similarly, serum protein (g/dl) and serum glucose (mg/dl) values were significantly ($P < 0.05$) influenced before and during the drug administration. However, the result of this study showed that all the does were in good health status after synchronizing oestrus with the drug.

Key words: Serum biochemistry, West African Dwarf (WAD) does, oestrus synchronization drug.

INTRODUCTION

Medroxy progesterone acetate (MPA) is an estrus synchronizing drug; a hormonal compound which has hormonal effects that are largely due to its ability to suppress the secretion of pituitary gonatropins that in turn prevent follicular maturation, thus producing long term anovulation in reproductive female.

The use of MPA to induce estrus in seasonal anaestrus and cycling ewes (Wildeus, 2004; Daniel et al., 2001) and does (Imasuen and Ikhimioya, 2009) has been well documented by several authors. However, its potential application on goats is yet to be fully explored, especially in our local breeds of goats such as West African Dwarf (WAD) does. Furthermore, there is the paucity of information on how the administration of this hormonal

drug may positively or otherwise interfere with the normal physiological process of the animal while trying to achieve oestrus manipulation with a view to harvest more kids from these animals.

Consequently, this experiment was conducted to assess the likely changes that may occur in serum metabolites of WAD doe administered MPA drug with the specific objective of ascertaining if this drug will cause any health problem in does administered varying doses of MPA drug.

MATERIALS AND METHODS

Description of location and site of experiment

The experiment was carried out at the Teaching and Research Farm, Faculty of Agriculture, Ambrose Alli University, Ekpoma in Edo State, Nigeria. The experimental site lies along latitude $6^{\circ}2$

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degree North and latitude 6 degree North located in the rainforest zone of Nigeria with an average annual rainfall of about 1500 to 2000 mm per annual and relative humidity of about 75% with a mean temperature of 24°C (Frederick et al., 2007).

Animals and management

Forty (40) cycling West African Dwarf (WAD) does used for this experiment were purchased directly from local farmers rather than buying from the open market so as to guarantee the quality of stock used.

Only does with four to six broad central teeth were purchased. This was done to ensure that only does that are cycling and between two to three years of age and weighing an average of 16.59 ± 0.76 kg as well as had kidded once or twice were used for the experiment. This was done using the dentition and weight range methods as outlined by Sastry and Thomas (1975) and Devendra and Mcleroy (1983). Prior to the commencement of the experiment, all does were quarantined for 30 days during which routine treatment developed by NAPRI (1984) was administered under the supervision of a veterinarian.

At the end of the quarantine period, the animals were randomly assigned to five treatment groups and each treatment group was assigned eight WAD does. The animals were separated into the treatment groups based on the route of administration and dosage of the drug administered. The five treatment groups consisted of the following: treatment 1: does administered 25 mg MPA (Depo - provera®) intramuscular injection, single dose; treatment 2: does administered 50 mg MPA (Depo - provera®) intramuscular injection, single dose; treatment 3: does administered 5 mg MPA (provera®) orally for ten days; treatment 4: does administered 10 mg MPA (provera®) orally for ten days; treatment 5: does administered 5 ml sterile water orally for ten days (this group served as the control group).

The dosage of medroxyprogesterone acetate (MPA) used in this experiment was as a result of the fact that this drug comes in an injectable form (Depo-provera®) and orally active tablets (provera®). The tablet form comes in 5 or 10 mg; therefore it was convenient to administer single tablets of 5 or 10 mg per day for 10 days. Also, the injectable form (Depo-provera®) was half the tablet form (provera®) because the injectable form is a long acting drug, which is always administered as a single dose.

The animals used for the experiment were identified individually with neck tags encoded according to treatment groups. All animals were managed semi-intensively. They were fed in the mornings between 8.00 and 10.00 h daily with *Arachis hypogea* (groundnut) hay, *Giliricidia sepium*, *Panicum maximum* and occasionally, *Zea mays* (maize) or *Manihot esculenta* (cassava) peelings, when available, before allowing them to go out from their pens into the adjoining fenced paddock. The animals were allowed to remain in the paddock to graze freely between the 10.00 and 18.00 h before allowed to return to their pens for confinement. Water and salt lick were provided *ad libitum*.

The experiment was carried out in three stages. The first stage of the experiment involved the acclimatization and stabilization of cycling does used for this experiment. Blood sample was collected from each experimental animal twice during this stage and the average of the result was used as pre-drug administration information.

The second stage lasted for two weeks during which the drug was administered and blood sample was also collected twice during drug administration, with the average of the result used as during-drug administration.

The third stage involved blood collection two weeks after drugs withdrawal from the does. Blood sample was also collected twice during the third and fourth weeks after drug withdrawal, and the average of the results was used as post-drug administration.

Determination of serum biochemistry

Total protein, albumin, globulin, glucose, creatinine and urea were determined using the methods described by Dacie and Lewis (1991), while sodium (Na^+) and potassium (K^+) were determined by flame photometry (Hawk et al., 1954).

Determination of serum enzymes activities

Serum alanine transaminase (ALT), serum aspartate transaminase (AST) and serum alkaline phosphatase (ALP) were analysed spectrophotometrically, using commercially available diagnostic kits (RANDOX® Test Kits).

Statistical analysis

All data collected were subjected to analysis of variance using the SAS/STAT (2004) package. Mean separations were done using the same software, where there were indications of significant differences Duncan's multiple range test was used.

RESULTS

The blood sera assessed in this experiment were serum electrolytes, serum enzymes activities and serum glucose and protein. These results are shown in Tables 1, 2 and 3. The result of the blood serum electrolytes showed that serum sodium (Na^+) and potassium (K^+) ions assessed were not significantly ($P > 0.05$) influenced by the drug administered within all the treatments, although significant differences ($P < 0.05$) existed between the control animals and other animals when K^+ was assessed between treatments during and after drug administration.

The level of urea in the blood serum showed that the drug administered significantly increased urea level during the period of drug administration within all the treatments, except in the control. In serum creatinine, the significant differences observed within and between the treatments did not follow any definite pattern and could therefore not be strictly attributed to the drug administered.

The result of enzymes activities in the blood serum as shown in Table 2, revealed that ALT and AST were not significantly ($P > 0.05$) influenced between and within all the treatments. ALP values showed significant ($P < 0.05$) decrease within treatments 2 and 4 after drug withdrawal. Similar significant ($P < 0.05$) differences were observed between treatments during drug administration.

Serum protein values as shown in Table 3 revealed that there were significant decrease ($P < 0.05$) within all the treatments post-drug administration, except in the control group. Similar increases were observed between the treatments during the drug administration.

Albumin and globulin values did not reveal any significant change ($P > 0.05$) during the drug administration. Conversely, glucose values were significantly ($P < 0.05$) influenced in treatments 3 and 4, as increase was observed during drug administration.

Table 1. Assessment of blood serum electrolytes in WAD doe administered varying level of MPA drugs.

Variable	Treatment				
	TRT 1 (25 mg Depo)	TRT 2 (50 mg Depo)	TRT 3 (5 mg Provera)	TRT 4 (10 mg Provera)	TRT 5 (Control)
Pre-drug administration					
Sodium (Na ⁺) (mEq/l)	141.00 ± 0.71 ^a _(x)	139.25 ± 1.25 ^a _(x)	139.50 ± 1.19 ^a _(x)	140.50 ± 1.19 ^a _(x)	140.00 ± 1.08 ^a _(x)
Potassium (K ⁺) (mEq/l)	4.50 ± 0.18 ^a _(x)	4.68 ± 0.47 ^a _(x)	4.50 ± 0.07 ^a _(x)	4.83 ± 0.43 ^a _(x)	4.33 ± 0.18 ^a _(x)
Urea (mg/dl)	22.50 ± 0.07 ^a _(z)	29.75 ± 4.76 ^a _(x)	23.00 ± 5.60 ^a _(z)	32.50 ± 3.23 ^a _(x)	26.50 ± 4.97 ^a _(x)
Creatinine (mg/dl)	0.65 ± 0.7 ^a _(y)	0.75 ± 0.13 ^a _(x)	0.78 ± 0.13 ^a _(x)	0.70 ± 0.06 ^a _(y)	0.65 ± 0.07 ^a _(x)
During-drug administration					
Sodium (Na ⁺) (mEq/l)	139.50 ± 0.65 ^a _(x)	140.50 ± 1.04 ^a _(x)	141.00 ± 0.41 ^a _(x)	139.50 ± 0.65 ^a _(x)	140.25 ± 0.95 ^a _(x)
Potassium (K ⁺) (mEq/l)	4.30 ± 0.18 ^{ab} _(x)	4.50 ± 0.22 ^{ab} _(x)	5.03 ± 0.21 ^a _(x)	4.83 ± 0.48 ^a _(x)	3.85 ± 0.05 ^b _(x)
Urea (mg/dl)	32.50 ± 2.96 ^a _(x)	30.75 ± 3.95 ^a _(x)	32.50 ± 2.33 ^a _(x)	31.50 ± 2.53 ^a _(x)	28.00 ± 3.08 ^a _(x)
Creatinine (mg/dl)	1.03 ± 0.05 ^a _(x)	0.80 ± 0.04 ^{ab} _(x)	0.90 ± 0.04 ^{ab} _(x)	1.00 ± 0.04 ^a _(x)	0.73 ± 0.08 ^b _(x)
Post-drug administration					
Sodium (Na ⁺) (mEq/l)	139.00 ± 1.08 ^a _(x)	140.75 ± 1.10 ^a _(x)	140.50 ± 0.87 ^a _(x)	139.0 ± 1.08 ^a _(x)	139.75 ± 1.37 ^a _(x)
Potassium (K ⁺) (mEq/l)	4.25 ± 0.25 ^{ab} _(x)	4.48 ± 0.19 ^{ab} _(x)	4.80 ± 0.36 ^{ab} _(x)	4.98 ± 0.36 ^a _(x)	3.93 ± 0.10 ^b _(x)
Urea (mg/dl)	29.50 ± 2.72 ^a _(y)	27.75 ± 3.57 ^a _(y)	28.25 ± 5.33 ^a _(y)	27.50 ± 3.07 ^a _(y)	27.00 ± 1.73 ^a _(x)
Creatinine (mg/dl)	0.70 ± 0.04 ^{ab} _(y)	0.87 ± 0.05 ^a _(x)	0.80 ± 0.11 ^{ab} _(x)	0.85 ± 0.14 ^{ab} _(y)	0.58 ± 0.05 ^b _(x)

a, b: Values with different superscript along the same row (between treatments) are significantly different (P < 0.05); x, y, z: values with corresponding parameters in parenthesis along the same column (within treatments) with different subscripts are significantly different (P < 0.05); TRT, treatment.

Other significant differences observed among the treatments did not follow any define pattern and could therefore not be tied to the drug administered.

DISCUSSION

Potassium ion (K⁺) is the major cation of the intracellular fluid and functions as sodium does in the extracellular fluid by influencing acid base balance (Adedeji, 1992; Guyton, 1985).

This mineral plays a vital role in muscle function. High blood levels of potassium are gene-

rally due to kidney failure or endocrine disease rather than from excessive dietary intake. There is a relationship between sodium and potassium metabolism (Campbell et al., 2003). Similarly, sodium ion is important for normal muscles contraction. It is also essential for maximum utilization of dietary energy and protein, and for efficient reproduction. On this premise therefore, potassium and sodium ion assessment in this study revealed that these two essential element were not significantly affected by the drug administered as all values obtain from the study fell within the range of 3.0 to 6.0 mmol/L for potassium ion and 124 to 146 mmol/L for sodium

ion as reported by Daramola et al. (2005) for WAD goats. However, report from temperate region shows a higher range of value for goats, sheep and cattle (Harper, 1982).

In this respect, WAD goats are probably similar to man and cattle which have been shown to have lower sodium level in tropical environment (Macfarlane et al., 1970; Oduoye and Fasanmi, 1971). This close association between tropical environment and lower sodium level in man has been attributed to variable dietary intake of salt and loss of sodium and chlorine ions in urine under tropical environmental condition (Macfarlane et al., 1970).

Table 2. Assessment of blood serum enzymes activities in WAD does administered MPA drugs.

Variable	Treatment				
	TRT 1 (25 mg Depo)	TRT 2 (50 mg Depo)	TRT 3 (5 mg Provera)	TRT 4 (10 mg Provera)	TRT 5 (Control)
Pre-drug administration					
ALP (mg/dl)	98.75 ± 22.72 ^b _(x)	187.00 ± 50.35 ^a _(x)	86.75 ± 11.38 ^b _(y)	117.25 ± 13.20 ^{a,b} _(x)	54.75 ± 16.84 ^b _(x)
ALT (μ/l)	10.25 ± 2.95 ^a _(x)	8.50 ± 1.32 ^a _(x)	9.00 ± 1.68 ^a _(x)	8.50 ± 2.10 ^a _(x)	11.00 ± 2.27 ^a _(x)
AST (μ/l)	62.75 ± 4.51 ^a _(x)	70.50 ± 10.57 ^a _(x)	69.50 ± 7.59 ^a _(x)	65.00 ± 4.25 ^a _(x)	56.25 ± 1.10 ^a _(x)
During-drug administration					
ALP (mg/dl)	93.25 ± 4.40 ^b _(x)	140.50 ± 34.51 ^a _(x)	117.00 ± 52.81 ^a _(x)	165.75 ± 43.27 ^a _(x)	75.50 ± 12.34 ^c _(x)
ALT (μ/l)	12.75 ± 1.10 ^a _(x)	9.00 ± 0.91 ^a _(x)	10.00 ± 1.08 ^a _(x)	11.75 ± 2.68 ^a _(x)	11.50 ± 1.19 ^a _(x)
AST (μ/l)	61.00 ± 2.48 ^a _(x)	65.25 ± 5.21 ^a _(x)	73.00 ± 5.84 ^a _(x)	67.75 ± 6.14 ^a _(x)	59.25 ± 3.06 ^a _(x)
Post-drug administration					
ALP (mg/dl)	79.25 ± 9.23 ^a _(x)	101.75 ± 15.29 ^a _(y)	105.00 ± 1.42 ^a _(x)	84.75 ± 4.75 ^a _(y)	78.25 ± 15.64 ^a _(x)
ALT (μ/l)	10.75 ± 1.25 ^a _(x)	8.50 ± 2.50 ^a _(x)	10.50 ± 1.04 ^a _(x)	11.50 ± 1.55 ^a _(x)	6.00 ± 1.68 ^a _(x)
AST (μ/l)	65.50 ± 3.81 ^a _(x)	63.25 ± 5.31 ^a _(x)	75.50 ± 6.70 ^a _(x)	67.75 ± 4.42 ^a _(x)	61.00 ± 5.11 ^a _(x)

a, b, Values with different superscript along the same row (between treatments) are significantly different ($P < 0.05$); x, y, values with corresponding parameters in parenthesis along the same column (within treatments) with different subscripts are significantly different ($P < 0.05$); TRT, treatment.

Urea and creatinine were the other blood metabolite assessed and the results showed that these metabolites were significantly increased ($P < 0.05$) during the drug administration and significantly decreased ($P < 0.05$) after drug withdrawal in all the treatment groups, except in treatments 2 and 3, where differences were not observed in creatinine value at the different stages studied.

The increase or decrease in the urea and creatinine values during drug administration and drug withdrawal agrees with previous findings of Sathyamorthy et al. (1981). They reported increase in serum urea and creatinine in rat. They reported that any endogenous toxic substance might manifest through reduced protein utilization, thereby increasing the catabolism of amino acid, which would be subsequently degraded into urea

and creatinine. Accurate determination of creatinine clearance is crucial to rational drug therapy because many drugs are either partially or totally eliminated by the kidney. Creatinine clearance is the most accurate test of renal function (Kassirer, 1971). The value of creatinine in this experiment was slightly below the value reported for goat and sheep (Schalm et al., 1975) but fell within the range reported for man (Adedeji, 1992).

It is a known and accepted fact that tissues soluble enzymes are very vital adjunct to clinical diagnosis of tissue damage and disease. Both ALT and AST in conjunction with other enzymes are indicators of liver and other body tissue damage (Retiman and Frankel, 1957; Rosalki and Wilkiron, 1976). ALT is involved in cellular

metabolism and energy processes of the cell, while AST is involved in the inter-conversion of glutamate to α -ketoglutarate in the cytoplasm and mitochondria of the cell. Consequently, changes in ALT and AST levels are indicators of liver damage and muscle necrosis (Rosalki and Wilkiron, 1976; Mitruka and Rawnsley, 1977).

In this study, serum alanine transaminase level was found not to be significantly increased during the course of drug administration and drug withdrawal. This is contrary to the report of Adedeji (1992) that oral administration of progestin-estrogen combination may elevate serum ALT and AST. Another factor that may elevate this serum enzymes activity is the plane of nutrition. Schimke (1962) stated that the levels of serum and liver ALT and AST frequently increase

Table 3. Serum proteins and glucose estimation in WAD does administered MPA drug.

Variable	Treatment				
	TRT 1 (25 mg Depo)	TRT 2 (50 mg Depo)	TRT 3 (5 mg Provera)	TRT 4 (10 mg Provera)	TRT 5 (Control)
Pre-drug administration					
Total Protein (g/dl)	7.80 ± .51 ^{bc} _(x)	6.07 ± 0.45 ^c _(y)	8.85 ± 0.78 ^{ab} _(x)	9.75 ± 0.47 ^a _(x)	7.75 ± 0.52 ^{bc} _(x)
Albumin (g/dl)	3.22 ± .35 ^a _(x)	3.67 ± 0.22 ^a _(x)	3.75 ± 0.15 ^a _(x)	3.92 ± 0.34 ^a _(x)	3.40 ± 0.08 ^a _(x)
Globulin (g/dl)	4.58 ± .67 ^a _(x)	2.40 ± 0.27 ^a _(x)	5.10 ± 0.86 ^a _(x)	5.83 ± 0.57 ^a _(x)	4.35 ± 0.49 ^a _(x)
Glucose (mg/dl)	44.75 ± 1.18 ^b _(x)	46.75 ± 3.77 ^b _(y)	60.25 ± 6.73 ^a _(y)	56.00 ± 5.55 ^{ab} _(y)	43.50 ± 1.44 ^b _(y)
During-drug administration					
Total Protein (g/dl)	7.15 ± 0.98 ^{bc} _(x)	7.52 ± .40 ^{abc} _(x)	8.95 ± 0.54 ^{ab} _(x)	9.50 ± 0.64 ^a _(x)	6.52 ± 0.35 ^c _(x)
Albumin (g/dl)	3.35 ± 0.51 ^a _(x)	3.57 ± .19 ^a _(x)	3.72 ± 0.25 ^a _(x)	3.90 ± 0.30 ^a _(x)	3.17 ± 0.41 ^a _(x)
Globulin (g/dl)	3.80 ± 0.47 ^b _(x)	3.95 ± .22 ^b _(x)	5.23 ± 0.47 ^a _(x)	5.60 ± 0.61 ^a _(x)	3.35 ± 0.13 ^b _(x)
Glucose (mg/dl)	49.50 ± 3.42 ^b _(x)	58.00 ± 6.17 ^{ab} _(x)	66.50 ± 8.92 ^{ab} _(x)	74.25 ± 1.75 ^a _(x)	53.25 ± 5.45 ^{2b} _(x)
Post-drug administration					
Total Protein (g/dl)	6.10 ± 0.15 ^a _(y)	6.60 ± 0.56 ^a _(y)	7.20 ± 0.23 ^a _(y)	7.17 ± 0.28 ^a _(y)	6.50 ± 0.33 ^a _(x)
Albumin (g/dl)	2.95 ± .15 ^a _(x)	3.22 ± 0.40 ^a _(x)	3.45 ± 0.29 ^a _(x)	3.40 ± 0.08 ^a _(x)	3.05 ± 0.9 ^a _(x)
Globulin (g/dl)	3.15 ± 0.08 ^a _(x)	3.38 ± 0.46 ^a _(x)	3.75 ± 0.31 ^a _(x)	3.77 ± 0.27 ^a _(x)	3.45 ± 0.9 ^a _(x)
Glucose (mg/dl)	49.00 ± 5.49 ^a _(x)	50.25 ± 3.56 ^a _(x)	56.75 ± 3.04 ^a _(y)	52.50 ± 7.08 ^a _(y)	48.00 ± 3.80 ^a _(xy)

a, b, c: Values with different superscript along the same row (between treatments) are significantly different (P < 0.05); x, y: values with corresponding parameters in parenthesis along the same column with different subscripts are significantly different (P < 0.05); TRT, treatment.

in response to decrease in energy intake while Ekpenyong and Biobaku (1986) reported that bone demineralization as a result of poor plane of nutrition may increase serum ALT and AST.

ALP constitutes a large group of isoenzymes, which plays important roles in the transportation of sugar and phosphate and originate from different tissues such as liver, bone, placenta and intestine (Adedeji, 1992). In normal adult, according to Adedeji (1992), the two main sources of ALP are bone and liver. Consequently, an elevation of alkaline phosphatase may be a result of either liver problem or bone disease. The same author also reported the elevation of serum alkaline

phosphatase as a result of administration of progestin-estrogen drug.

The significant difference (P < 0.05) observed in this study (Table 2) may therefore be attributed to the drug administered, possibly due to its metabolic effects in the liver and bones. It is however not certain why no effect was observed in treatments 1 and 3 during and after the drug withdrawal.

Plasma proteins serve as sources of rapid replacement of tissue proteins, as buffer in acid-base balance, and as transporters of constituents of blood such as vitamins, iron, copper, hormones, lipids and certain enzymes (Adedeji,

1992). About, 65% of the body total protein is albumin, which is produced by the liver, alongside globulin. Hansten (1980) reported that progestin-estrogen drug might elevate total plasma protein. Furthermore, the plane of nutrition has also been reported to affect total serum protein level (Bamgbose et al., 2004; Babatunde et al., 1992).

In this study, no significant effect was observed during the drug administration, except in treatment 2 where increase was observed. It seems therefore that the drug had a delayed effect as there was significant decrease in the level of serum total protein in post drug administration period for each treatment group in which the drug

was administered (Table 3).

Since the serum albumin and globulin levels were not significantly altered either during the drug administration, the difference observed in the total protein value could be attributed to the contribution of other protein sources in the blood.

Except in treatment 1 (Table 3), glucose value was significantly increased ($P < 0.05$) within treatments during drug administration. This increase seemed not to be related to the drug as similar increase was noticed in the control. For similar reason, it is difficult to explain the sustained increases observed among other treatments during the different stages of the experiment.

The observation in this case contradicts the report of Hansten (1980) who suggested that progesterone-oestrogen combination drug causes increase in the level of serum glucose. The contrary observation may be related to the absence of oestrogen in the drug; this is because synergistic effect of the progesterone and oestrogen cannot be ruled out.

Conclusion

Oestrus synchronization is one of the major scientific approaches currently employed to overcome the low reproductive ability of WAD goats as experienced in the traditional system of goat production in Nigeria. It was observed that administration of MPA drug could cause significant changes in body weight gain in WAD dose (Imasuen and Ikhimioya, 2009) and to some extent, alter the serum biochemistry of the animal.

Blood serum metabolite and serum enzymes such as urea, creatinine and alkaline phosphate evaluated during this experiment were found to be significantly ($P < 0.05$) influenced by the MPA drug administered, however, the degree was not such that was deleterious and therefore did not suggest ill health in the WAD does. Also, the significant ($P < 0.05$) increases observed in serum protein and glucose during the drug administration are all indication that MPA drug used in synchronizing oestrus could alter the overall physiological status of goats administered MPA drug.

Finally, the use of MPA drug in achieving oestrus synchronization in WAD does should be guided by the dose and rate of administering the drug so as not to cause any physiological disorder.

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