

Research Article





# Kidney function test, weight gain/ body conformation and serum protein values of mature balami rams treated with gonadotrophin (Diclair®) for sperm production

### **Abstract**

Twelve sexually matured (2-2.6 years old) healthy Balami rams which weighed between 45.55 and 45.57kg were used to determine the effect of Gonadotrophin (Diclair®) on kidney function, body conformation and serum protein values. The rams were divided into 4 groups of 4 animals per group with one ram per replicate in a Completely Randomized Design (CRD). These groups were assigned to 3 levels of pergonal as treatments. The levels of pergonal were 0.00i.u, 54.00i.u, 108.00i.u pergonal represented as T1 (control), T2 and T3 respectively. All the treatments were administered by intramuscular injections. The injections were divided into three doses each and administered intramuscularly in the thigh for three consecutive days. The results showed that there were significant differences (P<0.05) among the treatment groups in all the parameters for kidney function test: chloride, potassium, sodium, bicarbonate and creatinine. The results further showed that there were significant differences (P<0.05) among the treatment groups in final body weight, weight gain, and all the parameters for body conformation: heart girth, scrotal circumference, body length, hip width withers height except initial body weights which were similar (P>0.05) among the treatment groups. Similarly there were significant differences (P<0.05) among the treatment groups in all the serum protein values measured: albumin, globulin, serum total protein as well as globulin/ albumin ratio. The results of this study showed that Diclair® enhanced kidney function and weight gain without any deleterious effects on body conformation and serum protein values of the rams.

**Keywords:** Diclair®, kidney function, body conformation, serum proteins, Balami rams.

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## Introduction

The Balami sheep is the biggest of the Nigerian sheep breeds and is found mainly in the drier Sudan and Sahel Savannah zones. It has an all white coat, convex face with large and droopy ears and long tail. Males have horns while females are hornless. Mature males (rams) may have dewlap and weigh between 40-65kg, females weigh 30-45kg. The Balami sheep is also referred to as desert breed. It is a good dairy breed.1 In order to carry out any sustainable improvement in livestock, there should be methods of ensuring the repeatability and multiplication of desired traits in subsequent generations. Reproduction is a process by which an organism gives rise to a new member of its species. It is a vital factor in determining the efficiency of animal production and its performance is closely related to profitability in livestock enterprise.2 In view of the increasing use of livestock for specialized production, there is need for more practical and better control methods of reproduction. Sperm formation involves the use of follicle stimulating hormone (FSH) and luteinizing hormone (LH).3 Most of these preparations of FSH and LH are very expensive. Some of them require cold chain storage and often deteriorate because of inadequate storage and handling. 4 Diclair®, also known as Humegon or Mentrophin and with similar constituents as plusset® is a gonadotrophin preparation lyophilized in vials containing a mixture of follicle stimulating hormone and luteinizing hormone in a ratio 1:1.5 Follicle stimulating hormone and LH in Diclair® play vital

role in the initiation of spermatogenesis. The hormone preparation is cheap, readily available and does not require cold chain storage. It has not been determined if the administration of the hormone preparation for spermatogenesis and semen production would induce any side effects on the kidney function, body conformation and serum proteins values of the rams. This study was therefore carried out to determine the effect of Diclair®, administration on kidney function, body conformation and serum protein values of mature Balami rams.

# **Materials and methods**

### Experimental animals and their management

Twelve healthy, sexually matured Balami rams aged 2-3 years were used for this study. The animals were purchased from the local markets and housed in clean pens constructed in such a way that the rams could come outside during the day for access to sunlight and forage. The animals were deformed two times within the experimental period. The actual experimental period lasted for complete 70 days. Routine inspection for cleanliness was carried out. Freshly cut forage consisting of *Panicum* maximum, *Aspilia africana*, *Pennisetum purpureun* (Elephant grass) was fed as basal diet and a concentrate ratio of Grower Mash was used as supplement. The animals were fed twice daily, in the morning and evening. Salt lick was provided as mineral supplement. Water was given *ad libitum* to the animals.



### Experimental design and drug administration

The twelve Balami rams were divided into 3 experimental groups consisting of 4 animals per group with one animal per replicate in a Completely Randomized Design (CRD). These groups were assigned to 3 levels of Diclair® as treatments. The levels of Diclair® were 0.00i.u, 81.00i.u and 162.00i.u represented as T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. T<sub>1</sub>, which contained no Diclair® served as the control (Table 1). The rams were treated by intramuscular injection. The injections were given as follows:

Diclair® was supplied in 7 vails, each vial containing FSH 75i.u and LH 75i.u. The content of the first vial was dissolved in 1ml of physiological saline solution immediately prior to use, resulting in a solution of DFSH 75i.u plus DLH 75i.u per ml. All treatments were administered intramuscularly on the hind leg (thigh) of each ram using a one ml syringe with 0.01ml graduation.

Table I Doses of Diclair® administered to mature Balami Rams

Day	Treatment dosage (ml)			
	T,	T <sub>2</sub>	<b>T</b> <sub>3</sub>	
1	0	0.12	0.24	
2	0	0.12	0.24	
3	0	0.12	0.24	
Total	0	0.36	0.72	

### Blood collection and evaluation of blood chemistry

The rams were bled one week after Diclair® injections between 9am and 10.30am from punctured jugular vein and aspirated about 5ml of blood from each ram. The blood samples were poured into plain bottles and were allowed to coagulate to produce sera for blood chemistry analysis. The bottles of coagulated blood were subjected to standard methods of serum separation and the harvested sera were used for biochemical evaluation. The standard flame photometry using Gallenkamp analysis was used to determine serum sodium (Na<sup>+</sup>) ion and potassium (K<sup>+</sup>) ion. While bicarbonate and chloride ions were assayed according to the methods of Baker and Silverton.<sup>7</sup> Creatinine concentrations was also determined following methods described by Baker & Silverton.<sup>7</sup> Serum total protein was determined by Goldberg refract meter method as described by Kohn & Allen.<sup>8</sup> Albumin and globulin were determined using bromocresol green (BCG) method as described by Randox.<sup>9</sup>

### Body weight / body conformation measurements:

The body weights of the animals were measured in kg using a hanging scale. The rams were weighed every week and their weights were recorded. Sixty five days after Diclair® administration body conformation: heart girth, scrotal circumference, body length, hip width and withers height were measured in cm using a measuring tape (Table 2).

Table 2 Concentration of Diclair® on mature Balami Rams

Day	Concentration of diclair (i.u)			
	T,	<b>T</b> <sub>2</sub>	<b>T</b> <sub>3</sub>	
1	0	18	36	
2	0	18	36	
3	0	18	36	
Total	0	54	108	

### **Data analysis**

Data collected on kidney function test, body weight / body conformation and serum protein values of Balami rams were subjected to one-way analysis of variance (ANOVA) using the technique of Steel and Torrie. <sup>10</sup> Significant treatment means were separated using Duncan's New Multiple Range Test as described by Obi. <sup>11</sup>

### **Results and discussion**

The results of Diclai<sup>(R)</sup> administration on kidney function of mature Balami rams are shown in (Table 3). There were significant differences (P<0.05) among the treatment groups in all the parameters measured for kidney function test: Sodium, potassium, chloride, bicarbonate and creatinine. Rams on T<sub>3</sub> recorded the highest valve of 151.50 (mmol/L) in serum sodium and this differed significantly (P<0.05) from rams on T<sub>1</sub> which were similar (P>0.05) to rams on T, in sodium values. There was no significant difference (P>0.05) between rams on T<sub>3</sub> and T<sub>2</sub> in serum sodium values. The sodium values obtained in this study were within the normal range of 139-152 (mmol/L) reported in sheep by Kaneko et al. 12 Rams on T<sub>1</sub> recorded the highest value of 4.10 (mmol/L) in serum potassium and this differed significantly (P<0.05) from rams on T, which were similar (P>0.05) to rams on T<sub>3</sub> in potassium values. There was no significant difference (P>0.05) between ram son  $T_1$  and  $T_3$  in serum potassium values. The potassium values obtained in this study were within the normal range of 3.9-5.4(mmol/L) reported in sheep by Kaneko et al. 12 Potassium is excreted in the kidney and elevation of plasma potassium is indicative of under excretion suggesting kidney impairment. Rams on T, recorded the highest value of 101.50 (mmol/L) in serum chloride and this differed significantly (P<0.05) from rams on T<sub>1</sub> and T<sub>3</sub> which were similar (P>0.05) to each other in chloride values. The lowest value in serum chloride was observed in rams on T<sub>1</sub> (95.50 mmol/L). The serum chloride values obtained in this study were within the normal range of 95-103 (mmol/L) reported by Kaneko et al. 12 in sheep. Rams on T<sub>2</sub> recorded the highest value of 25.54 (mmol/L) in serum bicarbonate and this differed significantly (P<0.05) from rams on T, which were similar (P>0.05) to rams on T<sub>2</sub> in bicarbonate values. There was no significant difference (P>0.05) between rams on T<sub>3</sub> and T<sub>2</sub> in serum bicarbonate values. The serum bicarbonate values obtained in this study were within the normal range of 20-25 (mmol/L) reported in sheep by Kaneko et al. 12 Bicarbonate is used in the buffering system in the blood, extra cellular fluid and kidney (Brackett, 2005).<sup>13</sup> Rams on T<sub>3</sub> recorded the highest value of 167.00(mmol/L) in serum creatinine and this differed significantly (P<0.05) from rams on T<sub>1</sub> which were similar (P>0.05) to rams on T2 in creatinine values. There was no significant difference (p>0.05) between rams on T<sub>3</sub> and T<sub>2</sub> in serum creatinine values. The creatinine values obtained in this study were within the normal range of 106-168 (mmol/L) reported in sheep by Kaneko et al.<sup>12</sup> Creatinine measurement is used exclusively in the assessment, of kidney function. The rate of production of creatinine is constant and elevations of plasma creatinine are indicative of under excretion, suggesting kidney impairment.

Table 3 Effect of diclair® on kidney function of mature Balami Rams

Parameters treatment (Diclair® i.u)					
	T, 0.00	T <sub>2</sub> 54.00	T <sub>3</sub> 108.00	SEM	
Sodium (mmol/l)	146.50⁵	150.50ab	151.50°	0.82	
Potassium (mmol/l)	4.10 <sup>a</sup>	3.95 <sup>b</sup>	3.99 <sup>ab</sup>	0.04	
Chloride (mmol/l)	95.50⁵	101.50ª	96.50 <sup>b</sup>	1.86	
Bicarbonate (mmol/l)	20.025b	22.20ab	24.54 <sup>a</sup>	1.24	
Creatinine (mmol/l)	155.00 <sup>b</sup>	165.00ab	167.00 <sup>a</sup>	3.72	

a) The results of Diclair® administration on weight gain/body conformation of mature Balami rams are shown in (Table 4). There were significant differences (P<0.05) among the treatment groups in final body weight, body weight gain, scrotal circumference, heart girth, body length, hip width and withers height. However, there were no significant differences (P>0.05) among the treatment groups in initial body weight.

Table 4 Effect Of diclair® on weight gain/body conformation of mature Balami Rams

Parameters treatment (Diclair® i.u)						
	T, 0.00	T <sub>2</sub> 54.00	T <sub>3</sub> 108.00	SEM		
Initial body weight (cm)	45.55	45.56	45.57	0.01		
Final body weight (kg)	47.55⁵	47.96ab	48.27 <sup>a</sup>	0.21		
Body weight gain(kg)	2.00b	2.40 <sup>ab</sup>	2.70 <sup>a</sup>	0.20		
Scrotal circumference(cm) Heart girth (cm)	25.50 <sup>b</sup>	29.50 <sup>a</sup>	28.25ab	1.18		
	41.50ab	39.50 <sup>b</sup>	43.50ª	1.15		
Body length (cm)	68.45⁵	76.25 <sup>a</sup>	76.25ª	2.26		
Hip width (cm)	87.25 <sup>b</sup>	93.30 <sup>a</sup>	87.50ab	1.98		
Withers height (cm)	83.30 <sup>b</sup>	85.50 <sup>a</sup>	85.50 <sup>a</sup>	0.73		

- b) Rams on T<sub>3</sub> recorded the highest final body weight (48.27kg) and this differed significantly (P<0.05) from rams on T<sub>1</sub> which were similar (P<0.05) to rams on T<sub>2</sub> in final body weight. There was no significant difference (P>0.05) between rams on T<sub>3</sub> and T<sub>2</sub> in final body weights.
- c) Rams on T<sub>3</sub> recorded the highest body weight gain (2.70kg) and this differed significantly (P<0.05) from rams on T<sub>1</sub> which were similar (P>0.05) to rams on T<sub>2</sub> in body weight gain. There was no significant difference (P>0.05) between rams on T<sub>3</sub> and T<sub>2</sub> in body weight gain. The lowest body weight gain was observed, in rams on T<sub>1</sub> (2.00kg). The initial and final body weights obtained in this study were within the normal range of 40-65kg reported by Oni (2002) for Balami rams.
- d) Rams on  $T_2$  recorded the highest value of 29.50cm in scrotal circumference and this differed significantly (P<0.05) from rams on  $T_1$  which were similar (P>0.05) to rams on  $T_3$  in scrotal circumference values. There was no significant difference (P>0.05) between rams on  $T_2$  and  $T_3$  in scrotal circumference values. The lowest value in scrotal circumference was observed in rams on  $T_1$  (25.50cm).
- e) Rams on T<sub>3</sub> recorded the highest value of 43.50cm in heart girth and this differed significantly (P<0.05) from rams on T<sub>2</sub> which were similar (P>0.05) to rams on T<sub>2</sub> in heart girth values. There was no significant difference (P>0.05) between rams on T<sub>3</sub> and T<sub>1</sub> in heart girth. The lowest value in heart girth was observed in rams on T<sub>3</sub> (39.50cm).
- f) Rams on  $T_2$  and  $T_3$  recorded the highest value of 76.25cm in body length and this differed significantly (P<0.05) from rams on  $T_1$ . The lowest value in body length was observed in rams on  $T_1$  (68.45cm).
- g) Rams on  $T_2$  recorded the highest value of 93.30cm in hip width and this differed significantly (P<0.05) from rams on  $T_1$  which were similar (P>0.05) to rams on  $T_2$  in hip width values. There

- was no significant difference (P>0.05) between rams on  $T_2$  and  $T_3$  in hip width values. The lowest value in hip width was observed in rams on  $T_1$  (87.25cm).
- h) Rams on  $T_2$  and  $T_3$  recorded the highest value of 85.50cm in withers height and these differed significantly (P<0.05) from rams on  $T_1$ , which had the lowest value in withers height (83.30cm).
- i) The observation in this study that the rams on T<sub>3</sub> recorded the highest values in final body weight, body weight gain, heart girth, body length, and withers height suggests that the dose of the test drug (0.72ml Diclair®) given to rams on T<sub>3</sub> could have increased metabolism and efficient utilization of nutrients that resulted in improved body conformation and weight gain.
- j) The results of Diclair® administration on serum protein Values of mature Balami rams are shown in (Table 5). There were significant differences (P<0.05) among the treatment groups in all the serum protein Values measured: albumin, globulin, serum total protein and albumin/ globulin ratio.

Table 5 Effect of Diclair® on serum protein values of mature Balami Rams

Parameters treatment (Diclair® i.u)						
	T <sub>1</sub> 0.00	T <sub>2</sub> 54.00	T <sub>3</sub> 108.00	SEM		
Albumin (g/l)	24.16ª	20.10 <sup>b</sup>	20.26ab	1.33		
Globulin (g/l)	46.09b	52.15 <sup>ab</sup>	55.49ª	2.75		
Albumin/globulin Ratio	0.52a	0.36⁵	0.37 <sup>ab</sup>	0.05		
Serum total Protein (g/l)	70.25 <sup>b</sup>	72.25ab	75.75ª	1.61		

- k) Rams on  $T_1$  recorded the highest value of 24.16(g/L) in serum albumin and this differed significantly (P<0.05) from rams on  $T_2$  which were similar (P>0.05) to rams on  $T_3$  in serum albumin values. There was no significant difference (P>0.05) between rams on  $T_1$  and  $T_3$  in albumin Values. The serum albumin values obtained in this study were within the normal range of 24.0-30.0(g/l) reported by Kaneko et al.  $^{12}$  in sheep. Low albumin suggests poor clothing ability of blood and hence poor prevention of hemorrhage.  $^{14}$
- l) Rams on T<sub>3</sub> recorded the highest Value of 55.49 (g/L) in serum globulin and this differed significantly (P<0.05) from rams on T<sub>1</sub> which were similar (P>0.05) to rams on T<sub>2</sub> in globulin values. There was no significant difference (P>0.05) betweens rams on T<sub>3</sub> and T<sub>2</sub> in serum globulin values. The serum globulin values obtained in this study were within the normal range of 35.0-57.0 (g/L) reported in sheep by Kaneko et al.<sup>12</sup> Iheu kwumere et al.<sup>15</sup> inferred that a decrease in serum globulin is an indication of disease fighting ability of the body system and could lead to mortality.
- m) Rams on  $T_1$  recorded the highest albumin/ globulin ratio of 0.52 and this differed significant (P<0.05) from rams on  $T_2$  which were similar (P>0.05) to rams on  $T_3$  in albumin/globulin ratio. There was no significant difference (P>0.05) between rams on  $T_1$  and  $T_3$  in albumin/globulin ratio. The lowest albumin/globulin ratio was observed in rams on  $T_2$  (0.36).
- n) Rams on  $T_3$  recorded the highest value of 75.75 (g/L) in serum total protein and this differed significantly (P<0.05) from rams on  $T_1$  which were similar (P>0.05) to rams on  $T_2$  in serum total protein values. There was no significant difference (P>0.05) between rams

on  $T_3$  and  $T_2$  in serum total protein values. The serum total protein values obtained in this study were within the normal range of 60.0-79.0(g/L)reported in sheep by Kaneko et al. <sup>12</sup> Serum total protein is the protein retained in animal's body <sup>16</sup> Blood protein content has been shown to depend on the quantity and quality of dietary protein. <sup>16</sup>

### Conclusion

From the results of the study, it can be concluded that Diclair® improved kidney function and body weight gain / body conformation of Balami Yams at the level of 108.00 1.u without any deleterious effects on serum protein values.

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None.

# **Conflict of interest**

The author declares no conflict of interest.

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