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**EFFECT OF FORAGE RESTRICTION ON HAEMATOLOGY AND
SERUM MINERAL PROFILE OF WEST AFRICAN DWARF SHEEP**

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ABSTRACT

*The haematological and serum mineral profile of West African dwarf sheep following restricted feeding with *Panicum maximum* (PM), *Centrosema pubescens* (CP) and their combination (PM + CP) were evaluated in this study. Twenty ewes were randomly assigned to four treatment diets T1, T2, T3 and T4 in a completely randomized design (CRD) experiment of five animals per treatment. Sheep in T1 group received the control diet (100% free range forages) while T2, T3 and T4 received diets containing 100% PM, 100% CP and PM + CP (50%:50%) respectively. Forage restriction to PM alone when compared with free range feeding (control) produced a significantly ($p < 0.05$) higher mean RBC count, haemoglobin concentration (HbC) and PCV. The total leucocyte count, lymphocyte count, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly ($P < 0.05$) decreased following forage restriction to PM ($11.09 \pm 0.29 \times 10^3/\mu\text{l}$, $5.95 \pm 0.39 \times 10^3/\mu\text{l}$, $31.42 \pm 0.94\text{fl}$ and $10.82 \pm 0.40\text{pg}$ respectively). There was no significant difference ($p > 0.05$) in the mean neutrophil, basophil, monocyte counts and mean corpuscular haemoglobin concentration (MCHC) following forage restrictions. Forage restriction to PM had a significant ($p < 0.05$) decrease in mean serum calcium ($3.70 \pm 0.32 \text{ mg/dl}$) compared to CP ($5.04 \pm 0.46 \text{ g/dl}$) and PM+CP ($5.09 \pm 0.55 \text{ mg/dl}$) which were not significantly ($p > 0.05$) difference from each other. Serum phosphorus level also significantly ($p < 0.05$) decreased with forage restriction to PM and CP compared to PM+CP and the control which did not vary significantly ($p > 0.05$) from each other. It was therefore concluded from this study that forage restrictions of WAD sheep to PM or CP alone may likely predispose them to anaemia but very unlikely to concomitant deficiency of calcium or phosphorus.*

Keywords: Forage Restriction, Haematology, Serum, Minerals, Sheep

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INTRODUCTION

The greatest challenge to ruminant production, particularly in the tropics, is the provision of adequate nutrition especially during the dry season [1]. At this time, forages are relatively scarce and of poor quality especially with regards to protein and energy and therefore cannot adequately provide for maintenance let alone production. In order to meet the nutritional demands, farmers usually source feed(s) from various feed materials. However, under natural condition ruminants tend to limit themselves to available feeds within their immediate vicinity. Sometimes they are restricted to a particular feed resource or a combination of them in scarce quantity. Such restricted feeding conditions predispose the animal to certain physiological changes which may or may not support good performance and productivity.

Panicum maximum is a highly productive, palatable and well accepted grass that can be fed to ruminants alone or in combination with legumes. However, it has been reported that the crude protein content of the principal grasses in the savanna zone of Nigeria fall as low as 2% for most of the dry season, resulting in decreased voluntary intake and loss in weight in animals [2,3]. Lascana and Estrada [4] showed that voluntary intake in animals could be improved with protein deficiency and weight loss reversed significantly through the feeding of legumes in association with grasses. Consequently, under natural conditions, the nutritional value of *Panicum maximum* is usually improved by its combination with legumes such as *Centrosema pubescens* [4]. *Centrosema pubescens* is rich in protein which is usually the most limiting nutrient in tropical animal diets [5].

Evaluation of the blood profile of animals may give some insight as to the potentials of dietary treatments to meet the metabolic needs of the animals since according to Church *et al.* [5], dietary components have measurable effects on blood constituents such that significant changes in their values can be used to draw inference on the nutritive value of feeds offered to the animals. This work was therefore aimed at investigating the effect of forage restriction on haematology and serum mineral profile of West African dwarf sheep.

MATERIALS AND METHODS

Twenty adult sheep (ewes) weighing between 10 and 14kg and of 7 to 8 months of age were used for this study. They were housed in wooden metabolic crates in the Veterinary Teaching and Research Farm, University of Nigeria, Nsukka. The sheep were acclimatized for three weeks during which they were vaccinated against *pestes des petits ruminants* (PPR) using tissue culture PPR vaccine and dewormed using albendazole. The ewes were randomly assigned into four treatment diets T1, T2, T3 and T4 in a completely randomized design (CRD) experiment of five animals per treatment: the forages used for the study were *Panicum maximum* (PM) and *Centrosema pubescens* (CP) sourced from Sheep in the T1 group received the control diet (100% free range forages) while sheep in the T2, T3 and T4 groups received diets containing 100% PM, 100% CP and PM + CP (50%:50%), respectively. After a three week acclimatization period, the sheep were restricted to *ad libitum* feeding with the treatment diets in each group for ten weeks.

Blood samples (5 ml) were collected from each treatment group on the last day of the three weeks feeding interval by jugular vein puncture. One millilitre of blood dispensed into a sample bottle containing ethylene diamine tetraacetic acid (EDTA) was used to determine packed cell volume (PCV) [6], haemoglobin (Hb) concentration [7], white blood cell (WBC), total and differential counts [8] and red blood cell counts [8]. The remaining 4ml of blood were dispensed into plane test-tubes and was allowed to clot in a slanting position and centrifuged at 2,500 rpm for 5 minutes. The resulting sera were aspirated and used for serum calcium [9] and phosphorus [10] concentrations evaluations and calcium to phosphorus ratio was calculated. Study duration was 10 weeks.

Data generated were subjected to a one-way analysis of variance using a statistical package, SPSS version 16.0. Variant treatment means were separated using Duncan's Multiple Range Test [11].

RESULTS

Table 1 shows the effect of forage restriction on some haematological parameters of WAD sheep. Forage restriction to PM alone significantly ($p<0.05$) increased the RBC ($11.48\pm 0.78 \times 10^6/\mu\text{l}$) and HbC ($12.50\pm 1.12 \text{ g/dl}$) levels above those of all other forage groups and the control. The PCV was higher than those of the other forage groups but similar to that of the control. The total WBC and lymphocyte counts of the PM group were also significantly ($p<0.05$) lower than those of either the other forage groups or the control.

The MCHC level was similar ($p>0.05$) in all forage groups and the control. The MCV and MCH were also similar between the PM+CP forage group and the control but these were significantly ($p<0.05$) higher than those of either the PM or the CP forage restriction groups.

Table 1: Haematological parameters of West African dwarf sheep fed restricted forage diets for 10 weeks.

Parameter	Treatments			
	PM	CP	PM + CP	Control
RBC ($10^6/\mu\text{l}$)	11.48 ± 0.78^a	8.79 ± 0.25^b	8.44 ± 0.34^b	7.98 ± 0.26^b
HbC (g/dl)	12.50 ± 1.12^a	9.43 ± 0.51^b	10.55 ± 0.26^b	11.21 ± 0.24^{ab}
PCV (%)	36.08 ± 2.75^a	27.50 ± 1.43^b	30.83 ± 0.60^b	31.67 ± 0.99^{ab}
WBC ($10^3/\mu\text{l}$)	11.09 ± 0.29^a	13.53 ± 0.83^b	13.59 ± 0.83^b	13.76 ± 0.69^b
Neutrophil ($10^3/\mu\text{l}$)	4.15 ± 0.45^a	3.71 ± 0.37^a	3.99 ± 0.18^a	3.86 ± 0.19^a
aEosinophil ($10^3/\mu\text{l}$)	0.75 ± 0.20^a	0.00 ± 0.00^b	0.00 ± 0.00^b	0.07 ± 0.03^b
Basophil ($10^3/\mu\text{l}$)	0.02 ± 0.02^a	0.00 ± 0.00^a	0.05 ± 0.03^a	0.07 ± 0.03^a
Monocyte ($10^3/\mu\text{l}$)	0.23 ± 0.09^a	0.10 ± 0.05^a	0.10 ± 0.03^a	0.19 ± 0.07^a
Lymphocyte ($10^3/\mu\text{l}$)	5.95 ± 0.39^a	9.73 ± 0.51^b	9.46 ± 0.61^b	9.55 ± 0.54^b
MCV (fl)	31.42 ± 0.94^a	31.53 ± 2.27^a	36.83 ± 1.65^b	39.98 ± 2.06^b
MCH (pg)	10.82 ± 0.40^a	10.83 ± 0.83^a	12.60 ± 0.57^b	14.14 ± 0.56^b
MCHC (g/dl)	34.45 ± 0.89^a	34.30 ± 0.43^a	34.22 ± 0.56^a	35.48 ± 0.45^a

^{ab}Mean values in the same row with different superscripts are significantly different ($p<0.05$).

PM = *Panicum maximum* only; CP = *Centrosema pubescens* only; PM + CP = 50% *Panicum maximum* + 50% *Centrosema pubescens* and Control = Free range forage.

Table 2 shows the mean serum calcium and phosphorus concentrations and their ratios. Compared with the control ($5.57\pm 0.17 \text{ mg/dl}$), serum calcium was significantly ($p<0.05$) decreased during forage restriction to PM ($3.70\pm 0.32 \text{ mg/dl}$), but not to CP ($5.04\pm 0.46 \text{ mg/dl}$) and PM+CP ($5.09\pm 0.55 \text{ mg/dl}$). On the other hand, phosphorus concentration was significantly ($p<0.05$) decreased during forage restriction to PM (2.50 ± 0.15) and CP ($2.81\pm 0.27 \text{ mg/dl}$) but not to PM+CP ($5.67\pm 0.23 \text{ mg/dl}$) compared to the control ($5.86\pm 0.25 \text{ mg/dl}$). A calcium/phosphorus ratio of 1:2 was recorded during PM and CP forage restriction but 1:1 during PM+CP restriction and control respectively.

DISCUSSION

The PCV, RBC count and HbC which were significantly ($p<0.05$) increased following restriction to PM may probably be due to a relative sufficiency of dietary crude protein intake (6.65%), vitamin B12 and other minerals that support red blood cell formation in PM. This agrees with earlier report by Blood *et al.* [12] that attributed low PCV values to insufficient protein intake. Compared to the control group, out of all the differential leucocyte parameters investigated, only the lymphocyte count showed significantly ($p<0.05$) decreased during forage restriction to PM alone. This probably suggests that at this feeding standard (restriction to PM), the ability of the animals to in that group to maintain immunocompetence

was probably compromised. It is also possible that at this feeding regimen, the animals had decreased tolerance for certain stress factors such as infection, environmental stress (heat or cold) as well as nutritional stress which are capable of predisposing them to suppressed immunity. High environmental temperature has been shown to stimulate the thermal receptors to transmit suppressive nerve impulses to the appetite centre in the hypothalamus causing a decrease in feed intake [14]. Nutritional stress, on the other hand, leads to destruction of protein in tissues due to increase in glucocorticoid hormone responsible for protein catabolism [13]. This may affect the lymphoid tissues resulting in depleted lymphocyte population [13]. This mechanism probably accounted for decreased leucocytes populations observed in this study when the sheep were restricted to PM. It is also possible that infection, excitement and physical stress that are known to deplete lymphocyte populations may have contributed, in part, to the depleted lymphocyte populations in the PM fed sheep since all the animals experienced the same environment and handling during blood collection.

Table 2: The mean serum calcium and phosphorus concentrations and Ca:P ratio following 10 weeks of forage restrictions in WAD sheep.

Parameter	Treatments			
	PM	CP	PM + CP	Control
Calcium (mg/dl)	3.70 ± 0.32 ^a	5.04 ± 0.46 ^b	5.09 ± 0.55 ^b	5.57 ± 0.17 ^b
Phosphorus(mg/dl)	2.50 ± 0.15 ^a	2.81 ± 0.27 ^a	5.67 ± 0.23 ^b	5.86 ± 0.26 ^b
Calcium: Phosphorus	1:2	1:2	1:1	1:1

^{ab}Mean values in the same row with different superscripts are significantly different (p<0.05).

PM = *Panicum maximum* only; CP = *Centrosema pubescens* only; PM + CP = 50% *Panicum maximum* + 50% *Centrosema pubescens* and Control = Free range forage. Calcium : phosphorus ratio = the value of calcium divided by that of phosphorus.

Also the presence of traces of anti-nutritional factors in control diet might have triggered off immune response by lymphocytes which tended to rise. [14] reported that varying lymphocyte values indicate different levels of immune states of animals and Lazzaro [15] further reported that depressed levels of lymphocytes might indicate a depleted immune status in an active infection. However, lymphocyte counts obtained in this study falls within the normal physiological range of 40 – 75% for healthy sheep [16] which lays credence to the fact that the lymphocytic changes were more of dietary than any other exogenous effect.

As shown in Table 2, the serum calcium concentration of the control group was significantly (p<0.05) higher than that of PM but not for the other treatment groups. A similar finding was noted with phosphorus concentrations for PM and CP forage restrictions. The variation in the calcium and phosphorus levels in the serum of sheep on the different diets were probably due to variable intake of these diets and differences in concentrations of the minerals in the diets offered to them. Nutrient supply including minerals is higher when a wide range of forages are fed as was seen in the control. The calcium to phosphorus (Ca:P) ratio obtained in this study in all the forage restriction groups and control were within the normal range of Ca:P ratio (1:2.5) needed for optimal growth performance and was enough to avoid the predisposition of the animals to urinary calculi and osteoporosis.

CONCLUSION

It is evident from this study that restriction of WAD sheep to PM or CP alone could be associated with haematological changes characteristic of anaemia but that the calcium and phosphorus profiles of such animals may remain within normal physiological ranges.

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