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Short Communication

Performance and Parasitology of Semi-intensively Managed West African Dwarf Sheep Exposed to Gastrointestinal Helminth Infected Paddocks and Varied Protein-energy Feeds

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Abstract

Background: The performance and parasitology of semi-intensively managed West African dwarf (WAD) lambs were evaluated following exposure to gastrointestinal helminth infected paddock and varied protein-energy feeds.

Methods: Twenty four lambs obtained from the Department of Animal Breeding and Genetics and brought to Directorate of University farm (DUFARM) of Federal University of Agriculture Abeokuta, Ogun state, Nigeria, where the research was carried out in 2014, were grouped into four each containing six animals based on different energy-protein feed combination thus; group 1(G1) low energy low protein, group 2 (G2) low energy high protein, group 3 (G3) high energy low protein and group 4 (G4) high energy high protein. Experimental animals were supplemented with concentrate feed after grazing on daily in a nematode infected paddock. Clinical signs of infection were monitored. Live weight, faecal egg count (FEC), worm counts, packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cell count (RBC) were determined using standard methods.

Results: Anorexia and intermittent diarrhea were the observed signs. Worm counts did not differ significantly ($P=0.309$) among the groups. The weight and FEC differed significantly ($P<0.05$) across the days and among the groups, while haematological parameters increased significantly ($P<0.05$) across the days and among the groups.

Conclusion: Lambs in G2 followed by G4 showed improved parameters and superior performance when compared to the other groups. It is therefore recommended that feed high in protein content is capable of mitigating deleterious effect of gastrointestinal helminth parasitism.

Introduction

Gastrointestinal parasitism has been identified as a complex disease entity constituting a major impediment to efficient and profitable livestock production particularly in sheep and goats all over the world (1, 2). Parasites cause direct losses due to death and indirectly due to reduced productivity through reduced feed intake, decreased live weight gain and skin wool or mohair quality (3, 4).

There are various integrated approaches available for effective control of gastrointestinal parasites. These include use of anthelmintics, management of grazing land, proper stocking rate and appropriate rotational strategies (5). However, conventional and repeated use of anthelmintics as a means of worm control is now strongly questioned because of increasing development of resistance of parasites to the major anthelmintic groups (6, 7). Conversely, grazing management and biological control are non-chemical methods of parasite control that have proved to be effective (6).

Among the alternative methods of control currently available, is the manipulation of host. The breeding of resistant animals in order to improve the host resistance and/or resistance to parasite infestation or infection seems to represent one of the most promising options (8-11). Therefore, sheep that are well nourished will grow and reproduce faster and are better able to withstand the effects of worm infestation than those on low plane of nutrition (11). Research has shown that increased dietary intake of metabolisable protein and energy with high quality pasture can directly promote host resistance and resilience to worm infection (12). Protein energy deficiencies is a important cause of defective T cell function (13, 14) and T cells have been shown to play vital role in mediating acquired resistance to haemonchosis in sheep (13). Bowie (15) had shown the beneficial effect of treatment with cayenne pepper, garlic powder, and

diatomaceous earth on the packed cell volume and the faecal egg count of sheep infected with *Haemonchus contortus* while cassava foliage adequately reduced the adverse effects of gastrointestinal nematode infections in goats, in particular when offered as silage (16).

There have been previous attempts aimed at determining the effect of protein of various origins on gastrointestinal parasitism (17-19).

The relative importance of energy and the dominant effect of protein supply have been partly researched on in the development of resistance to infection (20). This has generated an argument on whether the control of helminths in livestock is more sensitive to protein than to energy. Therefore, the aim of this study was to investigate the effect of varied protein-energy combinations on clinical presentation, performance, haematology and adult worm load of gastro-intestinally parasitized, West Africa Dwarf lambs that have been managed semi-intensively.

Materials and Methods

Study Location

The study site is located in the rain forest vegetation zone of Southwestern Nigeria at latitude 7° 13', 49 N; longitude 3° 26', 11.98' E and altitude of 76 m above sea level. The climate is humid with a mean annual rain fall of 1037 mm and annual mean temperature and humidity of 43.7 °C and 82 respectively (Meteorology Department, Ogun – Osun River Basin Authority, Abeokuta, Ogun State, Nigeria).

Experimental Animals

Twenty-four newly weaned in 2014 and apparently healthy WAD lambs aged 5-6 months and weighing between 5 and 7 kgs were obtained from the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta.

Acclimatization and Pre-experimental Screening

The experimental animals were allowed thirty days of acclimatization prior to commencement of experiment. During this period, all animals were screened for gastrointestinal, ecto- and hemoparasites using standard protocols. Infected animals were treated with levamisole at 7.5 mg/kg (Chanelle Pharmaceuticals Ltd, Spain) and *Diminazīne aceturate* at 3.5 mg/kg (Vetindia Pharmaceuticals Ltd, India).

Housing and Feeding

Experimental animals according to group were tagged and housed in wooden pens with a slatted floor which ensured that animals did not had access to their faeces. All pens were netted with fly-proof nets and fly-proof aluminium netting placed underneath the slatted floor for total recovery of feces from each pen.

On day 1 of the experiment, the animals were grazed on nematode infected paddock of *Stylosanthus hamatus* and *Pennisetum pedicellatum* as identified by Pasteur and Rangeland Management Department of the Federal University of Agriculture, Abeokuta. This feeding pattern was supplemented by concentrate feed made from wheat offals, cassava and palm kernel cake in varying concentrations, thus grouping the animals into four groups. The crude protein and metabolisable energy contents of the feed supplement was determined as described by AOAC (21).

Experimental Design

Permission for this study was obtained from University Experimental Animal Ethics Committee through the College of Veterinary Medicine representative. The experiment was carried out according to international guiding principles for biochemical research involving animals (22).

The lambs were divided by complete randomized design (CRD) into four groups (G1, G2, G3 and G4) of six animals each. G1 received supplementary diet low in energy and protein, G2 low energy and high protein, G3 high-energy low protein and Group G4, high-

energy high protein. Experimental animals were initially allowed access to nematode infected paddocks for a period of 5 hours daily and thereafter fed with concentrate as described above (2.2.2). The experiment was conducted in a 90-day period.

Clinical Observations, Sample Collections and Processing

All groups were observed for clinical signs of gastrointestinal parasitism while faeces and blood were collected on days 0, 30, 60 and 90 of experiment. Body weight was similarly measured on those days.

The body weight was measured using a hanging scale (Votilia Company, Italy). 50 grams of faeces was obtained directly from the rectum after wearing a hand glove. This was used for determination of egg per gram of faeces by the aid of Mc master counting chamber (Pyser SGI Ltd, Whitlock Australia Co. Australia). Similarly, 5 ml of blood was collected from the jugular vein of individual animals into EDTA bottles. This was used for determination of hematological parameters (PCV, Hb and RBC) as described by Schalm et al. (23).

Humane Sacrifice and Determination of Worm Counts

At the end of the 90-day experimental period, the animals were humanely euthanized using Pentobarbitone sodium (Euthatal[®]) to determine the total number and type of worms harboured by individual animal.

Statistical Analysis

Data generated were analyzed using Statistical Analysis System (24) package version 8. Significant differences among groups and across days were tested using two-way analysis of variance (ANOVA).

Results

Clinical Observations

Lambs in G1 and 3 had anorexia by day 20 post infection and intermittent diarrhea by day

30 in five of the six lambs in either of the groups. However, only intermittent diarrhea was observed from day 60 in 5 out of six lambs in G2 and 4. The ages and weights of the experimental animals did not differ signifi-

cantly ($P < 0.05$) among the groups at the onset of experiment (Table 1). The % compositions of the concentrate diet fed the experimental animals are detailed in Table 2.

Table 1: Mean (\pm SD) pre-infection ages and body weights of WAD lambs fed varied energy- protein densities

Parameter	G1	G2	G3	G4
Age (months)	5.33 \pm 0.516 ^a	5.50 \pm 0.54 ^a	5.67 \pm 0.816 ^a	5.17 \pm 0.408 ^a
Weight (kg)	11.17 \pm 1.16 ^a	11.33 \pm 0.516 ^a	11.03 \pm 0.52 ^a	12.17 \pm 0.75 ^a

Same superscripts in rows did not differ significantly ($P > 0.05$) among the groups. Alphabetic marks represented significant variations in the parameters across the days and groups.

Table 2: Ingredient compositions (%) and result of proximate analysis of concentrate diet

Ingredients	G1	G2	G3	G4
Cassava	65.00	6.00	82.00	14.00
Wheat offal	33.00	78.00	8.00	36.00
Palm kernel cake	0.00	14.00	8.00	48.00
Bone meal	1.00	1.00	1.00	1.00
Oyster meal	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Calculated analysis				
Crude protein (%)	8.40	15.11	6.85	15.35
Metabolizable energy (MJ/Kg)	7.23	6.19	11.84	12.02

Keys: G1= Low Energy, Low Protein, G2= Low Energy, High Protein, G3= High Energy, Low Protein, G4= High Energy, High Protein

Parasitological and Haematological Parameters

Total and mean worm counts did not differ significantly ($P = 0.309$) among the groups (Table 3). Similarly, the species of gastrointestinal helminths recovered are presented in table 4. *T. columbriformis* was the most prevalent constituting the following; 44% in G1, 44% in G2, 57% in G3 and 43% in G4 (Table 4).

Table 5 contains the results of the mean body weight, egg per gram of faeces, RBC, PCV and Hb concentration of experimental animals during the experimental period. Mean weight of all groups increased significantly ($P < 0.05$) across days 30, 60 and 90 post infection. Among the groups, animals in G4 appeared to be heavier than G3 and 2 by day 90 post infection. However, the increase was not significant.

Table 3: Total adult worm and Mean (\pm SD) adult worm and fecal egg counts by day 90 post-infection of lambs fed varied energy protein densities

Day 90 post experiment		Total adult worms	Mean adult worm counts (SD)	Mean (SD) FEC
G1	n=6	176	35 \pm 25.7	183 \pm 16.67
G2	n=6	83	16.6 \pm 11.52	333.3 \pm 33.33
G3	n=6	105	21 \pm 22.16	666.6 \pm 105.4
G4	n=6	71	14 \pm 4.6	450 \pm 50

Table 4: No (%) distribution of parasites recovered at necropsy by day 42 post infection

Parasite	G1	G2	G3	G4
<i>Trichostrongylus columbianum</i>	77 (44.00)	36 (43.40)	59 (56.20)	31 (43.70)
<i>Haemonchus contortus</i>	63 (36.00)	20(24.09)	32(30.48)	14(19.70)
<i>Oesophagostomum columbianum</i>	26 (14.86)	18(21.68)	08(07.62)	14(19.70)
<i>Moniezia benedini</i>	09 (05.14)	02(2.40)	04(03.80)	07(09.86)
<i>Paraamphistomum cervi</i>		07(8.43)	02(0.90)	05(07.04)
Total	175(100.00)	83(100.00)	105(100.00)	71(100.00)

Among all groups, the FEC did not differ significantly ($P < 0.05$) on day 0, while significant ($P < 0.01$) but irregular pattern of change was observed on days 30, 60 and 90 within the groups. However, G1 was significantly ($P < 0.05$) higher on days 30 and 60 compared to the other groups. PCV of G4 significantly increased ($P < 0.05$) within the groups and across

the days of experiment. The RBC of experimental animals varied significantly ($P < 0.05$) on day 0 among the groups. This was followed by significant increase across the days in all the groups. Hb concentration did not vary among the groups on day 0, but showed significant increase ($P < 0.05$) within the groups for the entire experimental period.

Table 5: Mean (\pm SD) Weight, FEC, PCV, RBC and Hb of WAD lambs experimentally exposed to nematode infected paddock and fed with supplementary containing varied energy-protein concentrate diet

Parameter		Days Post Infection			
		Day 0	Day 30	Day 60	Day 90
Weight (kg)	G1	11.17 \pm 1.17 ^d	12.28 \pm 0.60 ^b	13.20 \pm 0.16 ^{ab}	14.30 \pm 0.19 ^a
	G2	11.33 \pm 0.52 ^d	13.10 \pm 0.24 ^c	14.78 \pm 0.24 ^b	16.60 \pm 0.22 ^a
	G3	11.03 \pm 0.05 ^d	11.93 \pm 0.21 ^c	14.20 \pm 0.18 ^b	15.70 \pm 0.19 ^a
	G4	12.17 \pm 0.75 ^d	14.12 \pm 0.37 ^c	16.20 \pm 0.31 ^b	18.30 \pm 0.19 ^a
FEC (epg)	G1	400.00 \pm 89.44 ^c	2300.00 \pm 63.25 ^b	3333.33 \pm 210.82 ^a	183.33 \pm 16.67 ^d
	G2	350.00 \pm 83.67 ^c	1350.00 \pm 22.36 ^a	650.00 \pm 50.00 ^b	333.33 \pm 33.33 ^c
	G3	366.67 \pm 81.65 ^c	950.00 \pm 22.36 ^a	1066.67 \pm 42.16 ^a	666.67 \pm 105.41 ^b
	G4	366.67 \pm 81.65 ^c	500.00 \pm 44.72 ^b	850.00 \pm 22.36 ^a	450.00 \pm 50.00 ^{bc}
PCV (%)	G1	26.67 \pm 1.37 ^b	28.17 \pm 0.31 ^{ab}	30.17 \pm 0.83 ^a	30.33 \pm 1.15 ^a
	G2	26.33 \pm 2.73 ^b	27.67 \pm 0.56 ^{ab}	29.83 \pm 1.25 ^a	30.33 \pm 0.96 ^a
	G3	26.33 \pm 2.66 ^b	26.17 \pm 1.08 ^{ab}	31.33 \pm 1.82 ^a	32.17 \pm 1.92 ^a
	G4	26.50 \pm 2.26 ^b	28.00 \pm 1.34 ^b	30.17 \pm 1.91 ^a	34.67 \pm 0.42 ^a
	*(Ref:	24.9+1.95)			
RBC (x 10 ¹² /l)	G1	9.75 \pm 0.48 ^{bc}	9.58 \pm 0.16 ^c	10.37 \pm 0.21 ^b	11.35 \pm 0.33 ^a
	G2	9.88 \pm 0.26 ^b	9.62 \pm 0.18 ^b	10.18 \pm 0.24 ^b	13.58 \pm 0.35 ^a
	G3	8.97 \pm 0.41 ^b	9.43 \pm 0.22 ^c	10.67 \pm 0.32 ^b	12.78 \pm 0.40 ^a
	G4	9.55 \pm 0.25 ^b	9.83 \pm 0.15 ^b	10.18 \pm 0.28 ^b	13.88 \pm 0.38 ^a
	*(Ref:	8.17+ 1.28)			
HB (g/dl)	G1	8.45 \pm 0.15 ^c	9.25 \pm 0.11 ^b	9.93 \pm 0.34 ^{ab}	10.43 \pm 0.27 ^a
	G2	8.55 \pm 0.34 ^c	8.92 \pm 0.20 ^b	9.38 \pm 0.35 ^b	10.58 \pm 0.14 ^a
	G3	8.25 \pm 0.22 ^c	9.00 \pm 0.17 ^b	11.02 \pm 0.31 ^a	10.55 \pm 0.36 ^a
	G4	8.32 \pm 0.12 ^c	9.40 \pm 0.11 ^c	10.43 \pm 0.31 ^b	11.42 \pm 0.20 ^a
	*(Ref:	7.38 + 1.90)			

Different superscripts in columns and across rows differ significantly ($P < 0.05$). * Reference values adapted from Olayemi et al. (39) / Alphabetic marks represented significant variations in the parameters across the days and groups.

Discussion

The need to minimize production losses in animal husbandry practices is of paramount importance especially among young animals where ability to thrive and function optimally is threatened by infections like gastrointestinal helminths.

The diarrhea observed in all the groups in this study is an evidence of parasitic gastroenteritis (25). This is especially important among young animals such as those used in this experiment. *T. colubriformis*, an important contributor to parasitic gastroenteritis complex accounted for between 43 and 57% of the total worms recovered at necropsy in all the groups and might have been an important contributor to the diarrhea observed. This finding agrees with previous report by Chiejina (26) who associated diarrhea in sheep with the non-blood sucking helminths such as *T. colubriformis*. Such helminths cause proliferative gastrophly, catarrhal inflammation and epithelial ulcerations, thereby resulting in morphologic and functional damage of the gastro-intestinal tract. This subsequently leads to reduced absorption of water and electrolyte and finally diarrhea as a consequence (26, 27). Similarly, the anorexia observed in this study was obvious by day 20 post infection in G1 and G3 lambs fed on low protein diets. In ruminants, cobalt deficiency and a heavy infestation with trichostrongylids are common causes of anorexia (25). This is further corroborated by the previous finding of Steel (28) who observed reduced feed intake when FEC was above 3000 epg in sheep infected with trichostrongylids. Animals in G1 and 3 had FEC of (3333.33 ± 210.82) and 1066.67 ± 42.16 at day 60 post infection respectively. The absence of anorexia in groups (G2 and 4), that received high plane of protein is an indication of the ability of high protein diet to mitigate the negative impact of infection. Increased availability of protein has been reported to reduce the degree of anorexia in sheep with *T. colubriformis* infection (20). In addition, Kayriazakis et al. (29) attributed re-

duction in appetite in helminth-infected lambs to be immunostimulatory in origin, which allow for selective feeding particularly for high protein diets. This is necessary because of priority sequestration of amino acid by gastro intestinal tract for repairs, development and expression of local cell mediated responses. This might explain why lambs in G1 and G3 with low protein diets showed obvious anorexia, with high FEC at the peak of infection. In all groups, weight changes showed significant ($P < 0.05$) increase despite significant increase in FEC especially from days 30 and 60. The improved weight of animals in G4, followed by G2 compared to other experimental animals attest to the importance of protein and energy diets in improving productivity despite the effect of gastrointestinal parasitism. This finding agrees with the earlier report (19), in WAD goats experimentally infected with *H. contortus* and *T. colubriformis* and supplemented with dietary protein. Balanced energy-protein supplementation might have afforded opportunity for rumen to function optimally; thus enhancing microbial flora synthesis leading to increase in weight. A 60% increase in protein content of diet was associated with 50% increase in growth rate while 20% increase in energy content resulted in doubling of growth rate over ten weeks period in weaned Merino sheep infected with black scour worms (30). The same phenomenon might have been responsible for the significant weight gain in all groups especially those on high plane of protein and energy. McLure et al. (31) observed a strong negative correlation between an initial weight and *T. colubriformis* worm count after challenge in 8-month-old Merino sheep.

The significant decrease in FEC observed at the latter part of this experiment in all groups indicate that the effect of energy protein supplementation in enhancing immune response appears to be most effective at the latter stages of parasitic acquisition by the host. This observation corroborates an earlier report (20), where effect of protein supplementation be-

came manifest 15 weeks post infection. However, the significant decrease in FEC on day 90 post experiment in G1 and G2 compared to G3 and G4 could show the detrimental effects of low energy content on fecundity or oviposition of higher worm load. This is in agreement with the report of Marume et al. (18) on Xhosa lop-eared goats fed supplementary *Acacia Karroo* leaves following experimental haemonchosis. This decrease in FEC as seen G1 and G2 with low energy diets in this study was found to be incompatible with research into roles of energy in immune response to gastro-intestinal helminth parasitism (32-34). The lower FEC reported in G1 and G2 with higher worm load and conversely higher FEC reported in G3 and G4 with lower worm burden demonstrate positive effects of protein supplementation on worm load. The effects of low energy may have affected negatively on the fecundity of worm in the infected animals. Contrary to research stating that reduced FEC in lambs provide a selection criterion for worm resistance, which has been found to be heritable characteristic (35-37), the present study thus showed that, the use of reduced FEC in lamb for worm resistance might not be true in animal fed on low energy diet.

The PCV of all groups did not vary significantly ($P < 0.05$) as the experiment progressed except in G4. This absence may mean that there is constant supply of precursors necessary for erythropoiesis. The provision of additional protein to infected animals are able to maintain normal rate of growth suggesting that, if protein supply is high enough, then, it may possible to keep animal performance in the face of larval challenge (38). The recovery of blood sucking helminths such as *H. contortus* in low number compared to non-blood sucking helminths is a possible explanation for the ability of the animal to maintain normal PCV throughout the experimental period. Equally, the reasons adduced for the improved PCV may hold true for RBC and Hb concentration. Furthermore, the presence of parasites such as

M. benedini and *P. cervi* in low number, compared to others might have been responsible for the parameters observed. This is because such parasites do not cause much damage, unless present in large number, which was not the case in this study.

Conclusion

Diet low in energy and high in protein as in G2 supplemented lambs appear to be the most preferred diet combination in mitigating the deleterious effects of gastrointestinal helminth infection. This is approved by lower adult worm count and reduced FEC in the group.

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