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Effect of White Rot Fungi (*Pleurotus tuber-regium*) degraded Cassava Peels on Haematological Indices of Lactating West African Dwarf (WAD) Sheep.

*G.O. Ochepo[1], J. A. Ayoade[1], S. Attah[1] And A. Y. Adenkola[2]
[1]Department of Animal Production, University of Agriculture, Makurdi, Benue State, Nigeria.
[2]Department of Physiology, Pharmacology and Biochemistry, University of Agriculture, Makurdi, Benue State, Nigeria.

Abstract: *A trial was conducted to study the effect of White rot Fungi (Pleurotus tuber-regium) degraded cassava peels (FDCP) on haematological indices of lactating WAD ewes. Fifteen lactating WAD ewes, arranged in a completely randomized design, with 5 treatments and three replicates, were used. The treatments comprised; a control diet (T_1) containing 32 % un-degraded cassava peels (UDCP) and Four (4) graded levels of FDCP diets; T_2, T_3, T_4 and T_5 with 8%, 16%, 24% and 32% levels of inclusion, respectively. The experimental animals were each fed 500 g of the diets between 8:00 – 12:00 hours and thereafter allowed access to natural pasture dominated by A. tectorum throughout the period of the trial. Blood samples were collected from the experimental animals at 2, 6, and 12 weeks postpartum and subjected to haematological and serum protein analyses. The result showed that the PCV values (26.47-39.23 %) were generally within the normal range (27-43 %) for sheep. The Red Blood Cells count (RBC) values (8.68-13.78 $x10^{12}$/l) were similar to the normal range (9-14$x10^6$/ul) for sheep. In week 2, the hemoglobin (HGB) levels were significantly ($P < 0.05$) higher for ewes on T_2 (10.97 g/dl) and T_5 (10.75 g/dl) compared to ewes on T1, T_3 and T_4 with values of 8.27, 8.27 and 10.07 g/dl, respectively. In week 6, the HGB values were still significantly ($P < 0.05$) different. However, at week 12 there was no significant ($P > 0.05$) difference in HGB level across the treatments. The total protein values (62.33 - 75.77 g/l) recorded in this study is similar to the normal range (60 -75 g/l) for sheep. The albumin values (30.67 – 48.60 g/l) observed in this study was higher than the normal range (22 – 30 g/l) reported for sheep. It was observed that feeding of BDCP as a supplementary diet did not adversely affect the health status of the experimental animals. It is therefore concluded that FDCP is safe for feeding lactating WAD sheep.*

Keywords: *un-degraded, degraded, cassava peels and haematological indices*

I. Introduction

Poor and inadequate nutrition have been widely implicated among the constraints facing livestock production in developing countries [26]. Cattle, sheep and goats obtain daily nourishment by gleaning on natural forage made up of grasses, legumes, browse plants etc, whose quality and relative abundance are influenced by season and soil type. Grasses, the most abundant feed resource cannot meet the nutritional requirement for maintenance, growth and production in ruminants, when fed sole, because it contains low crude protein and age tends to increase its crude fibre concentration [5]. The basal feed resources for ruminants available in most developing countries in the tropics are crop residues, forages from infertile land or agro industrial by-products [16]. These are low in protein and of low digestibility.

The primary production and the processing of agricultural products have led to availability of various by-products. A huge tonnage of cassava peels is produced annually in Nigeria. A good percentage of this is set on fire while a fraction is utilized as animal feed, and on many occasions, left in the farm to rot. These lignocellulose by-products, however, have a low feeding value. In feed technology, relatively simple and cheap pre-treatments using alkali, sulphide, steam, mechanical and fungi treatments are being tried in order to improve the digestibility or the feeding value of the product so that it can be fed effectively to ruminants. It has been observed that the lignocellulose potential of the cellulose is encrusted by lignin within the lignocellulose matrix. .A combination of solid state fermentation (SSF) technology with the ability of an appropriate fungus to selectively degrade lignin will make possible industrial scale implementation of lignocellulose-based biotechnologies [18]. Digestibility of lignocellulose (fibrous crop residues) is known to be correlated with lignin content. In order to improve the digestibility of lignocellulose, physical, chemical and biological methods of delignification can be used [4][14]. White-rot fungi, such as edible mushrooms, can degrade fibrous crop residues and by-products, thereby increasing its nutritional value. Edible mushrooms are able to bioconvert a wide variety of lignocellulosic materials due to the secretion of extra cellular enzymes [9]. Various authors [6][17] have observed that the bioconverted materials have higher content of protein, a decreased fiber value and can be used as ruminant feed supplement. The incorporation of fungal-treated wastes along with other feeds in the diet of small ruminants can offset the dry season shortages of forages.

Haematology is the study of the natural functions and diseases of blood while haematological indices are those factors in the blood which are usually evaluated in order to assess the health status of an animal. The physiological importance of erythrocytes in domestic livestock has prompted studies that led to the establishment of some indices with which the health and performance of the animals can be monitored [3]. According to [2], more important among the health conditions is anaemia which can be monitored using indices such as packed cell volume (PVC), red blood cell (RBC) count, haemoglobin (Hb) contents, mean corpsular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Packed cell volume is the most accurate, simple and inexpensive method for the detection of degree of anemia. Packed cell volume less than normal is an indication that the animal is anemic. Estimating white blood cells (WBCs) total number and each type (neutrophils, lymphocytes, monocytes, basophils and eosinophils) is helpful in disease diagnosis. Leucopenia is a decrease in the number of circulating leukocytes and can be associated with infections of viral origin that destroy actively dividing blast cells of bone marrow [8]. Previous studies have shown that changes in haematological index variables can be an indicator to various disease and deficiency conditions [23] [10]. Albumin is an important protein which is 35 -50% of the total protein in serum or plasma. It is a major transport protein in blood which maintains osmotic pressure of plasma [8]. Hypoalbuminemia or decrease in albumin is seen in malnutrition.

This study was conducted to evaluate the effect of feeding graded levels of *Pleurotus tuber-regium* degraded cassava peels diets on the heamatological indices and serum proteins of lactating ewes.

II. Methodology

Experimental site

The experiment was carried out at the Livestock Teaching and Research Farm of University of Agriculture, Makurdi. Makurdi is the capital of Benue State and is located on longitude 8^0 37^1 East and latitude 7^0 41^1 North, with annual rainfall ranges from 609.9 mm – 1219.8 mm, temperature ranges from 25.6^0 C -39.6^0 C and relative humidity of about 21 % - 85 % [25].

Fungal Treatment of Cassava Peels (on-farm condition)

Cassava peels were obtained from a 'gari' processing mill in Makurdi and sun-dried for a period of seven days to reduce the moisture content to less than 10 %. The dried cassava peel was milled using a hammer mill. Five hundred kilograms (500kg) of milled cassava peels were moistened with water on a concrete floor and covered with cellophane sheet and allowed to ferment for two weeks as described by [1].

Pleurotus tuber-regium (edible mushroom) was purchased from Makurdi Modern Market and soaked in water for 24 hours in a basin. The wet mushroom was then transferred into a clean basin and allowed to grow mycelia. After the completion of the composting process, the fermented substrate was transferred to inoculation trays (60 cm x 180 cm) and allowed to cool for about one hour; the trays were subsequently inoculated with the active mycelia of the mushroom as described by [1]. At the end of the fermentation period of 30 days, the treated cassava peels were sundried until the substrate attained less than 10 % moisture content. The dried treated cassava peels were bagged and stored until required for feeding trials with West African Dwarf (WAD) lactating ewes.

Experimental Animals and their Management

Fifteen lactating WAD ewes, in a completely randomized design comprising three ewes per treatment, were used in a five-treatment feeding trial to study the influence of fungi-treated cassava peel on haematological indices. The animals were screened for blood parasites and treated with Diminazine aceturate before the commencement of the study. Diet T_1 contained 32 % un-degraded cassava peels (UDCP) and served as the control, while diets $T_{2,}$ T_3, T_4 and T_5 contained 8%, 16%, 24% and 32% cassava peels degraded with *Pleurotrus tuber-regium* (FDCP), respectively. Five hundred grammes (500 g) of each experimental diet was fed to each ewe in designated animal groups daily from 8:00 – 12:00 hours and thereafter allowed access to natural pasture dominated by *Andropogon tectorum*. Blood samples were collected from the three ewes on each experimental diet via jugular vein-puncture at weeks 2, 6, and 12 postpartum for haematology and serum protein analyses. Blood samples (5 ml / animal) were collected with 5 ml syringe and 2.5 ml placed into sterile vacutainer tubes containing ethylene diamine tetracetic acid (EDTA) for haematological study, while the remaining 2.5 ml was discharged into sterile vacutainer tubes without EDTA to allow blood clotting and serum decant for analysis.

Packed cell volume (PCV) and erythrocyte counts were determined as described by [11]. Total leukocyte counts were determined using Neubauer haemocytometer after appropriate dilution. Blood constants (Mean cell volume, MCV, Mean cell haemoglobin, MCH and Mean cell haemoglobin concentration, MCHC) were determined using appropriate formulae as described by [15]. Serum total protein was determined using

Biuret method as described by [22]. Albumin was determined using bromoscresol green (BCG) method as described by [21]. Serum creatinine was determined as described by [24]. Data obtained were subjected to one - way analysis of variance (ANOVA) using Minitab Statistical Software [19].

III. Results And Discussion

The proximate composition of un-degraded and fungi degraded cassava peels are shown in Table 1, while the proximate nutrient composition of the experimental diets is presented in Table 2. The range of 95.15 – 95.3 % DM reported for all the diet showed that the feeds were dried. The CP content ranged between 16.38 and 18.88 % in the five treatment diets which is a reflection of the level of FDCP in the diets. FDCP contained 6.04 % CP, thus, increased proportion of FDCP resulted in increased CP in the diets. The CF content on the other hand decreased with increased proportion of FDCP in the diets, which is as a result of the bio-degradation of the cassava peels. The haematological and biochemical parameters of ewes fed experimental diets at week 2, 6 and 12 are shown in Tables 3, 4 and 5, respectively. The pack cell volume (PCV) values ranged from 26.47 – 34.67, 30.70 – 39.23 and 33.30 – 38.70 percent at week 2, 6 and 12, respectively. There was no significant difference ($P > 0.05$) for pack cell volume (PCV) at week 2 across the treatments. However, at week 6 there was a significant difference ($P < 0.05$) in the PCV values between the ewes fed the control diet and ewes fed diet 5 which has 32 % FDCP. The PCV values recorded in this study were generally within the normal range (27-43 %) for sheep. Packed cell volume less than normal is an indication that the animal is anemic. The Red Blood Cells (RBC) values : 8.68-12.11, 9.34 – 13.62 and 11.70 – 13.78 $x10^{12}/l$ recorded in week 2, 6 and 12, respectively were within the normal range ($9\text{-}14x10^{6}/ul$) reported by [8]. This is an indication that the experimental animals maintained a good health status throughout the period of the study as reflected in the values for mean corpuscular hemoglobin concentration (MCHC): 308 – 320, 295-341 and 309-318 g/l and mean corpuscular hemoglobin (MCH): 9.03-9.55, 8.77-9.63 and 8.20-9.50 Pg, recorded at week 2, 6 and 12, respectively, which were also within the normal levels for sheep. Mean cell haemoglobin concentration is very significant in the diagnosis of anaemia and index of the capacity of the bone marrow to produce red blood cells [22].

The hemoglobin (HGB) levels range from 8.27 – 10.97, 9.03 – 12.00 and 10.30 – 12.20 g/dl at week 2, 6 and 12, respectively. In week 2, the hemoglobin (HGB) levels were significantly ($P < 0.05$) higher for ewes on T_2 (10.97 g/dl) and T_5 (10.75 g/dl) compared to ewes on T_1 and T_3 with a value of 8.27 g/dl. The values for T_1 and T_3 are slightly below the normal range for sheep (9-14 g/dl). In week 6, the HGB levels were still significantly ($P < 0.05$) different with ewes on T_1 having the lowest value. At week 12, there was no significant ($P > 0.05$) difference in HGB level across the treatments. These suggest that the test diets have no debilitating effect on health of the ewes. The white blood cell counts even though were significantly ($P < 0.05$) different at weeks 2 and 12, the values were within the normal range reported by [8] for sheep. This is an indication that the animal did not face any serious health challenge throughout the period of the experiment.

The total protein values recorded ranged from 67.33 – 75.77, 62.33 -74.00 and 71.60 – 74.90 g/l at week 2, 6 and 12 respectively. The total protein values were significantly ($P < 0.05$) different at week 6 with ewes on the control diet (T_1) having the lowest value (2.33 g/l). However, the values are within the normal range (60 -75 g/l) for sheep [8]. The albumin levels ranged from 33.00 – 38.37, 30.67 – 44.40 and 34.30 – 48.60 g/l at week 2, 6 and 12 respectively. The values were significantly ($P < 0.05$) different at week 6 and 12. Albumin is an important protein which is 35 - 50% of the total protein in serum or plasma. It is a major transport protein in blood which maintains osmotic pressure of plasma [8]. Hypoalbuminemia or decrease in albumin is seen in malnutrition. The albumin values observed in this study were higher than the normal range (22 – 30 g/l) reported for sheep [8]. This could be an indication of good protein metabolism preceded by proper nutrition [13]. The creatinine levels ranged from 2.40 – 7.20, 2.23 – 3.40 and 1.5 – 2.10 mg/dl at week 2, 6 and 1, respectively. Creatinine levels of 7.2, 3.6, 2.55, 2.43 and 2.4 mg/dl observed for T_1, T_2, T_3, T_4 and $T_{5,}$ respectively at week 2, decreased significantly ($P < 0.05$) with the inclusion of FDCP in the diets at week 2. These values are above the normal range for sheep (1.2 - 1.9 mg/dl). It was reported by [7] that a major source of excess creatinine in the blood of animals is when muscle wasting occurs and creatinine phosphate is catabolised. This suggests that the experimental animals were on a low plane of nutrition prior to the time of this study. At week 12, the creatinine levels dropped to the normal range except for the ewes on T_3 (2.1 mg/dl) which was slightly above normal. Creatinine is formed from creatine which stores energy in muscles in the form of phosphocreatine. Report by [8] shows that when the physical activity of the body is normal, the creatinine in blood remains within normal range; this suggests that the test diets were nutritionally adequate to maintain the normal physical activity of the body of the experimental animals.

Table 1: Proximate Composition of Un-degraded and Fungi degraded Cassava Peels (%) (DM)

	UDCP	FDCP	SEM
Dry Matter	93.70^{b}	94.70^{a}	0.091
Crude Protein	5.41^{b}	6.04^{a}	0.006
Crude Fibre	15.40^{b}	17.40^{a}	0.091
Ether Extract	0.65^{b}	1.10^{a}	0.028
Ash	15.60^{b}	19.20^{a}	0.091
Nitrogen Free Extract	56.64^{a}	50.96^{b}	0.055
Calcium	0.62^{b}	0.75^{a}	0.012
Potassium	0.05^{a}	0.004^{b}	0.004

^{ab} Means on same row with different superscripts differ significantly (P<0.05)

UDCP = Un-degraded cassava Peels

FDCP = Fungi degraded cassava peels

Table 2: Proximate Composition of Experimental Diets (%) (DM)

	T_1	T_2	T_3	T_4	T_5	SEM
CP	16.38	17.56	17.89	18.22	18.88	0.018
CF	13.88	13.64	13.39	13.15	12.90	0.015
EE	4.23	4.21	4.19	4.18	4.16	0.001
Ash	13.95	14.24	14.53	14.81	15.10	0.010
NFE	51.56	50.35	50.00	49.64	48.96	0.146
Ca	1.78	1.79	1.80	1.81	1.82	0.006
P	0.42	0.42	0.41	0.41	0.41	0.001
DM	95.20	95.20	95.30	95.30	95.15	0.015
GE MJ/kg	1.721	1.721	1.717	1.713	1.711	

SEM = Standard error of mean

FDCP= Fungi degraded cassava peels

T_1= Diet containing 0% FDCP, T_2= Diet containing 8% FDCP, T_3= Diet containing 16% FDCP, T_4= Diet containing 24% FDCP, T_5= Diet containing 32% FDCP

Table 3: Hematological and Biochemical Values (at Week 2) of Sheep Fed Experimental Diets

	T_1	T_2	T_3	T_4	T_5	SEM
PCV (%)	26.47	34.67	26.83	32.10	33.60	1.554
Platelet($x10^9$/dl)	824	621	381.67	409.67	541.50	87.254
HGB (g/dl)	8.27^{b}	10.97^{a}	8.27^{b}	10.07^{b}	10.75^{a}	0.448
RBC ($x10^{12}$/l)	8.68^{b}	12.11^{a}	9.10^{b}	11.02^{ab}	11.26^{ab}	0.502
MCV (Pg)	30.78	28.87	29.60	29.17	30.15	1.096
MCH (Pg)	9.50	9.20	9.03	9.07	9.55	0.260
MCHC (g/l)	312	320	307.67	313	319	3.765
WBC ($x10^9$/l)	11.05^{ab}	10.30^{ab}	7.93^{b}	12.37^{ab}	14.50^{a}	1.070
Neutrophils (%)	35	38.67	37	41.67	36.50	3.666
Lymphocytes	60.33	58.33	58	54.33	58.00	4.331
Eosinophils	0.10	01.33	4.67	3.67	5.00	1.483
Monocytes	0.40	0.20	0.33	0.33	0.50	0.640
Basophils	0	0	0	0	0	0
Total proteins (g./l)	67.33	75.77	75	73.57	74.85	6.073
Albumin (g.l)	33	38.37	35.80	37.23	36.15	3.131
Creatinine(mg/dl)	7.20^{a}	3.60^{b}	2.55^{b}	2.43^{b}	2.40^{b}	0.612

^{ab} Means on the same row with different superscripts differ significantly (P<0.05)

FDCP= Fungi degraded cassava peels

T_1= Diet containing 0% FDCP, T_2= Diet containing 8% FDCP, T_3= Diet containing 16% FDCP, T_4= Diet containing 24% FDCP, T_5= Diet containing 32% FDCP

Table 4: Hematological and Biochemical Values (at Week 6) of Sheep Fed Experimental Diets

	T_1	T_2	T_3	T_4	T_5	SEM
PCV (%)	30.70^{b}	37.67^{ab}	37.70^{ab}	35.90^{ab}	39.23^{a}	1.426
Platelet($x10^9$/dl)	651.67	606.33	479	525	492	69.633
HGB (g/dl)	9.03^{b}	11.60^{a}	11.60^{a}	10.67^{ab}	12.00^{a}	0.345
RBC ($x10^{12}$/l)	9.34^{b}	12.93^{a}	13.02^{a}	12.07^{a}	13.62^{a}	0.417
MCV (Pg)	32.8	29.27	29.00	29.73	28.87	0.720
MCH (Pg)	9.63	8.97	8.90	8.80	8.77	0.202
MCHC (g/l)	295	341.33	307	297	305.67	9.888
WBC ($x10^9$/l)	9	10.40	11.80	11.40	13.03	1.051
Neutrophils (%)	35	44	50	44	43.33	2.928
Lymphocytes	59.67	54	41	53.33	53.33	3.997
Eosinophils	01	2.67	07	1.33	2.67	1.249
Monocytes	4.33	1.33	03	1.33	0.67	0.841
Basophils	0	0	0	0	0	0
Total proteins (g./l)	62.33^{b}	74^{a}	71.20^{ab}	71.33^{ab}	72.10^{ab}	1.841

Albumin (g.l)	30.67^{b}	38.93^{ab}	44.40^{a}	38.17^{ab}	39.30^{ab}	1.668
Creatinine (mg/dl)	2.23	2.63	3.40	2.90	2.87	0.437

^{ab} Means on the same row with different superscripts differ significantly (P<0.05)

FDCP= Fungi degraded cassava peels

T_1= Diet containing 0% FDCP, T_2= Diet containing 8% FDCP, T_3= Diet containing 16% FDCP, T_4= Diet containing 24% FDCP, T_5= Diet containing 32%FBDCP

Table 5: Hematological and Biochemical Values (at Week 12) of Sheep Fed Experimental Diets

	T_1	T_2	T_3	T_4	T_5	SEM
PCV (%)	36.60	35.50	37.90	33.30	38.70	0.772
Platelet (x10^9/dl)	473	511	376	498	520	56.127
HGB (g/dl)	11.50	11.30	11.80	10.30	12.20	0.657
RBC (x10^{12}/l)	12.17	13.78	13.10	11.70	12.97	0.870
MCV (Pg)	30.30^{a}	25.80^{b}	29.00^{a}	28.50^{a}	29.90^{a}	0.445
MCH (Pg)	9.50	8.20	9.00	8.80	9.40	0.416
MCHC (g/l)	314	318	311	309	315	4.803
WBC (x10^9/l)	10.30^{ab}	11.30^{a}	9.40^{b}	10.50^{ab}	9.10^{b}	0.276
Neutrophils (%)	38^{b}	44^{ab}	53^{a}	43^{ab}	47^{ab}	2.218
Lymphocytes	56^{a}	47^{a}	30^{b}	50^{a}	46^{a}	1.514
Eosinophils	04^{b}	05^{b}	12^{a}	04^{b}	05^{b}	0.936
Monocytes	02^{b}	04^{a}	05^{a}	03^{ab}	02^{b}	0.347
Basophils	0	0	0	0	0	0
Total proteins (g./l)	74.90	72.40	74.20	71.60	72.00	0.798
Albumin (g.l)	37.10^{b}	39.00^{b}	48.60^{a}	38.10^{b}	34.30^{b}	1.933
Creatinine (mg/dl)	1.80	1.70	2.10	1.80	1.50	0.096

^{ab} Means on the same row with different superscripts differ significantly (P<0.05)

FDCP= Fungi degraded cassava peels

T_1= Diet containing 0% FDCP, T_2= Diet containing 8% FDCP, T_3= Diet containing 16% FDCP, T_4= Diet containing 24% FDCP, T_5= Diet containing 32% FDCP

IV. Conclusion

Feeding of FDCP as a supplementary diet did not adversely affect the health status of the experimental animals. It may therefore be concluded that FDCP is safe for feeding lactating WAD sheep.

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