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SUPPLEMENTING CONCENTRATE DIET WITH CABBAGE (*Brassica oleracea*) AND LETTUCE (*lactuca sativa*) LEAVES AS ROUGHAGE SOURCES AND THEIR IMPACT ON THE HAEMATOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF GROWING RABBITS

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ABSTRACT.

This trial was conducted to study the effects of supplementing concentrate with discarded cabbage (*Brassica oleracea*) and lettuce (*Lactuca sativa)* foliage on the blood characteristics of growing rabbits. A proximate composition of the cabbage and lettuce were carried out before the commencement of the feeding trial. In the sixty (60) days feeding trial involving forty (40) growing rabbits aged between 6 and 8 weeks with average initial weight of 750 g. The rabbits were allotted to four experimental treatments in a completely randonmised design to carry out a comparative study between the performance of growing rabbits fed discarded *Brassica oleracea* and *Lactuca sativa* leaves in combination with concentrate diet and those fed solely on concentrate diet. Treatment 1 were fed the control diet, which is made up of concentrate only. Treatments 2 and 3 were fed diet containing discarded cabbage and lettuce leaves in combination with concentrate respectively. Treatment 4 were fed diet containing an equal mixture of cabbage and lettuce leaves in combination with concentrate. Freshly collected *Brassica oleracea* and *Lactuca sativa* leaves were offered to the animal *ad libitum* and concentrate was given at 3% of their body weight. Treatment 2, 3 and 4. The chemical composition of *Brassica oleracea* and *Lactuca sativa* leaves showed that lettuce contained a higher crude protein (21.20%)) Fat (2.8%) Moisture (15.07%) and ash (21.60%) While cabbage is higher in crude fibre (7.65%) and energy (2182.20) than lettuce. The haematological and biochemical parameters investigated showed significant variations in the MCH, MCHC and Neutrophils and in glucose, HDL, TC, TG and ALT respectively. All these fell within the normal physiological range values given by established authorities and were comparable to values obtained by other researches within the tropics. It was concluded that the feeding of green cabbage and lettuce chop with concentrate given at 3% of the rabbit's body weight led to good performance. The result suggest that supplementing concentrate with cabbage leaves could be used without any detrimental effect on the performance of rabbits.

Keywords: Blood performance, rabbit, concentrate cabbage lettuce

INTRODUCTION

Coping with the dual challenge of higher feed prices and increased health concerns in animal production, the pressure on farmers and feed producers is also increasing as the demand for healthy animal products at reasonable prices is ascending steadily (Hoffmamn, 2008).The blood plays a vital role in the transportation of nutrients, metabolic waste products and gases around the body. The major objective of livestock production is to provide safe and healthy animal food/protein for the growing population (Shukla *et al.,* 2013). Blood components are affected by many factors like age, sex, physiological status, health, nutrition and breed or genotype/ Great variation has been observed in the *haematological* parameters not only among different animal species but between breeds of the same species, these differences have underlined the need to establish appropriate physiological baseline values for various breeds of livestock in Nigeria (Daramola *et al*., 2005). Chineke *et al*. (2006) also reported that apart from genotype, age and sex, differences in *haematological* indices may be caused by nutritional factor. *Haematological* studies represent a useful process in the investigation of the extent of damage to the blood. Reports by different researchers indicated that different diets fed to rabbits had different effects on *haematological* parameters, some of which were detrimental while others improved their *haematological* indices as they remained within the normal range of values for rabbits (Etim *et al*., 2014) Studies investigating the effect of feeding concentrates in combination with grass and legume on performance of grower rabbits have been carried out. Nigeria (Plateau state) is endowed with cabbage and lettuce by-products that are firstly derived from processing of food for human consumption or discarded for poor quality and or damaged during harvest and this have been used for animal feeding (Usman and Shaahu, 2023) The objectives of this study were to determine the effects of discarded cabbage and lettuce leaves and their mixture on the *haematological* and biochemical parameter of weaned rabbits

MATERIALS AND METHOD

Experimental Location

The experiment was conducted in the Federal College of Veterinary and Medical Laboratory Technology (FCVMLT) Animal Experimental farm Vom. In Plateau state. Vom town is situated in Jos South Local Government of Plateau State in Nigeria's middle belt. The main occupation of the people is farming and rearing of animals (Mangai, 2017).

Experimental Design, Animals and Management

Forty (40) weaned rabbits, comprising of male sexes and weighing 700g on the average and 6-8 weeks of age sourced from the same location of the study were allocated into four (4) experimental treatments in a Completely Randomised Design (CRD) with ten (10) rabbits per treatment each treatment having five (5) replicates. Two animals constituted a replicate and each replicate were housed in a separate hutch raised from the ground. Each hutch were provided with aluminum feeders and drinkers for daily provision of feed and fresh water. The animals were kept for one week acclimatization. Initial and weekly weights was taken before feeding in the morning (7.00-8.30 am) weekly, the quantity of feed supplied to them was weighed every morning and the left over from the previous day's feed were weighed and recorded to determine feed intake. The experimental diets were offered *ad libitum* in separate metal feeders in the morning (08.30h), so the rabbits determine their intake of the feed. Each rabbit were fed and assigned diet for 8 weeks (60 days).

Experimental Diet Preparation and Feed formulation

The Cabbage and Lettuce leaves discards were collected the previous day washed, air dried and given to the animal at 8:00 h daily. The forage were provided *ad libitum.* The experimental diet were formulated as shown in table 1. The rabbits were divided into 4 treatment groups i.e. T_1 (Concentrate Diet only), T_2 (Concentrate + Cabbage leaves) T_3 (Concentrate + Lettuce leaves). And T_4 (Concentrate + Cabbage + Lettuce). During the feeding time the animal were given the concentrate ration and subsequently the Cabbage and Lettuce leaves or their mixtures for those in group 2, 3 and 4. Water was provided *ad libitum***.** All the feed ingredients including discarded cabbage and lettuce leaves were obtained from Jos and environs.

Table 1: Gross composition of experimental Diet

Ingredient	**%**
Corn	21.20
Maize bran	44.00
Soya bean Cake (SBC)	18.00
Wheat offal	12.25
Fish meal	2
Salt	0.5
Premix	0.25
Methionine	0.25
Bone meal	1.5

Haematological **and serum biochemical analysis**

At the eighth (8) week blood samples of the rabbits from all the treatments were collected from the jugular vein into designated labeled tubes and used for *haematological* and serum biochemical analysis

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) Significant differences and among means was separated by Duncan's multiple range tests using the statistical Product and service solutions (SPSS) version 23

RESULT AND DISCUSSION

Table 2: Effects of Concentrate, Cabbage and Lettuce Leaves roughages on the *Haematological* Characteristics of Growing Rabbits

	Dietary Treatments						
Parameters	T1	T2	T3	T4	SEM	p-value	LOS
HCT (%)	42.90	45.20	39.70	35.08	1.37	0.211	NS
RBC (10^{12}/L)	8.26	6.61	8.01	5.43	0.5	0.144	NS
WBC (10^9/L	6.52	8.83	6.73	8.05	0.48	0.302	NS
HGB (g/L)	120.30	119.50	117.00	122.00	2.76	0.953	NS
MCV (fL)	54.30	68.50	51.70	65.70	3.37	0.214	NS
MCH(pg)	15.00^b	18.07^b	15.03^b	22.87^a	1.12	0.009	*
MCHC (g^2/L)	280.67^b	261.67^b	295.33^b	345.67^a	10.53	0.003	*
Neutroph (%)	23.00^{ab}	22.00^{ab}	31.70^a	12.70^b	2.92	0.135	*
Lymph (%)	74.70	76.00	64.70	81.30	3.06	0.300	NS
Monocytes	2.33	2.00	3.33	0.33	0.51	0.214	NS

[a, b, c & d] means with different superscripts within each row are significantly different at $p<0.05$.
T1= Concentrate only, T2 = concentrate + cabbage T3= Concentrate + lettuce, T4= Concentrate +Cabbage + Lettuce HCT=Heamatocrit RBC=Red blood cell, WBC=White blood cell MCV=Mean corpsular volume MCH=Mean corpuscular Heamoglobin MCHC=Mean Corpuscular heamaglobin concentration HGB=Heamoglobin Lymph=lymphocytes Neutroph=Neutrophils

Haematological Characteristics of Growing Rabbits

The haematological components of rabbits studied are shown in Table 2. The feeding of sole concentrate and concentrate supplementation with cabbage and lettuce green leaf chop to rabbits did not show significant ($p>0.05$) variation in the Hematocrit (HCT), white blood count (WBC), lymphocytes, monocytes, red blood count (RBC), haemoglobin concentration (HGB) and mean corpuscular volume (MCV) but showed significant ($p<0.05$) variations in the, Neutrophils, mean corpsular haemoglobin (MCH) and mean corpsular haemoglobin concentration (MCHC). The Neutrophils values range is (12.7 to 31.70) and MCH (15.0 to 22.87pg) with T4 been significantly higher than the other treatments while the MCHC (261.67 to 345.67g/L), T4 was significantly higher than the other treatments

Mean corpuscular heamoglobin (MCH) and mean corpuscular concentration (MCHC)

Njidda *et al*. (2006) posited that MCV, MCH and MCHC are used in diagnosing anaemic conditions. The feeding of the various dietary treatment did not show any significant difference on MCV and the range obtained (51-68.5fL) is between the normal range of (58-67 fL) reported by Duncan and Prasse, (1984). The MCH were significantly affected ($P<0.05$) with the range of (15-22.87pg) This range is not too far from that which was posited by Medirabbit (2007) and Duncan and Prasse, (1984) which reported 18-24og and 17-24 pg respectively but disagreed widely with the report by MS_2 Pulm Normal Labs (2017) that reported 26-34pg as the normal range for MCH. Slight differences in values of such can be attributed to test methodology and conditions of assay, units and variety of additional circumstances. A low value MCH typically indicates the presence of iron deficiency and anemia but in other cases low MCH can be caused by a genetic condition called thalassemia. The MCHC showed a significant difference ($P <0.05$) and the range of values obtained in this study (261.67-345.67g/L) was similar to the values of (27-37 g/dL) reported by Duncan and Prasse, (1984). MCHC results are specifically looked at when symptoms of anemia are present or when looking for the different causes of anemia especially when the RBC and/or haemoglobin levels are low but this study shows that both the RBC and heamoglobin levels fall within the normal values as reported by Medirabbit (2011)

Neutrophils

The 12.7 to 23 % range obtained in this study for neutrophils is lower than the values of (37.66 to 41.72%) obtained by Kagya-Agyemeng (2013) and 34 to 70 % reported as normal range by Medirabbits, 2007. Neutrophil are a type of white blood cell that help heal damaged tissues and resolves infections. Neutrophils level can rise or fall in response to infections, injuries, drug treatments, certain genetic conditions and stress (Medical News Today, 2023). The higher neutrophils values for T4 compared to other treatment could be due to genetic conditions and stress and sometimes due to unknown reasons called chronic idiopathic neutropenia and the abnormalities in the spleen (Mellillo, 2007). The lymphocytes values obtained from this study (64.7-81.3 %) is higher than the values reported as the normal physiological ranges for rabbits (25-

50 %)Jenkins, 1993;Hillyer, 1994;Nuhu, 2010) and the values (35.89-38.10%) reported by Kagya-Agyemeng (2013), this may be an indication of an underlining bacterial infection (Mark Sharp and Dohme Corporation, 2011) as the primary role of the lymphocytes is to respond to those activities that stimulate the immune system. (Melillo, 2007). The monocyte obtained from this study is in the range of (0.3-3.33) and this is within the normal range (0-4 %) reported by medirabbits. (2011).

Table 4: Biochemical Parameters of Growing Rabbits fed

Concentrate, Cabbage and Lettuce Diets

Dietary Treatments							LOS
Parameters	T1	T2	T3	T4	SEM	p-value	
Total Protein (g/I)	14.17	12.83	17.07	13.03	0.97	0.435	NS
Globulin (mmmoI/I)	5.3	3.7	6.33	4.4	0.44	0.164	NS
Albumin (g/I)	4.5	4.03	8.a	4.4b	1.62	0.831	NS
Glucose (mmmoI/I)	5.37[a]	5.40[a]	5.33[a]	3.93[b]	0.22	0.013	*
HDL (mmmoI/I)	1.87[a]	0.90[b]	1.2b[b]	0.83[b]	0.13	0.001	*
TC (mmoI/I)	4.43[a]	3.57[b]	4.07[ab]	3.37[b]	0.16	0.038	*
T.G (mmoI/I)	0.67[b]	0.83[b]	1.40[a]	1.23[a]	0.10	0.007	*
ALP (iu/I)	24.33	24	26	28.33	1.00	0.454	NS
ALT (iu/I)	23.67[ab]	20.33[b]	28.33[a]	22.00[ab]	1.24	0.097	*
AST (iu/I)	25.33	28.67	27	25	1.38	0.824	NS

[a, b] means with different superscripts within each row are significantly different @ $p>0.05$.
*= ($p<0.05$). NS= Not significant T1= Concentrate only, T2 = concentrate + cabbage T3= Concentrate + lettuce, T4= Concentrate +Cabbage + Lettuce TC=Total cholesterol TG= triglycerides, HDL=high density lipids ALP=alkaline phosphate ALT=alanine aminotransferase AST=aspartate aminotransferase

Biochemical Characteristics of Growing Rabbits

The serum biochemical components of rabbits studied are shown in Table 12. The feeding of sole concentrate and concentrate supplementation with cabbage and lettuce green leaf chop to rabbits did not show significant ($p>0.05$) variation in the albumin, total protein, ALP, AST and globulin but showed significant variations in glucose, HDL, total cholesterol, TG, and ALT. the value for glucose in this study range from3.93 to 5.5.37 with T1 to T3 significantly higher than T4. And the TG also showed significant variations among treatments where T3 and T4 are significantly higher that T1 and T2

Biochemical Components

Glucose

Glucose metabolism in rabbits is different from dogs or cats. Rabbits eat continuously during the day and also use volatile fatty acids produced by cecal flora as a primary energy source. A fasting blood sample is impossible to obtain because rabbits ingest fecal pellets. A rabbit that is not given food can continue to ingest cecotrophs. It has been shown that 4 days of starvation does not reduce blood glucose levels in rabbits (Melillo, 2007). Blood glucose according to Harcout-Brown (2012) is a measurable parameter that can be used to assess the severity of a rabbit's condition and help to differentiate between gut stasis and intestinal

obstruction in rabbits that are anorexic. The value of glucose observed from this investigation was significantly different ($P < 0.05$) with the range of (3.93-5.40 mmol/l) and this fell within the range of 4.2-8.9 mmol/l reported by medirabbits, (2012). T4 has a lower glucose level than the rest of the group. Higher glucose level can be perfectly normal in a young rabbit whereas in adult it would indicate stress or pathology such as G I disease. Gluscose values are also reported to present diurnal variations and affected when animals are frightened when handled or restrained without anesthesic (Melillo, 2007)

Total cholesterol (TC), triglycerides (TG), and high density lipids (HDL)

Cholesterol is synthesized in the liver or obtained from the diet and is a precursor of steroids, cholesterol and triglycerides levels peak after a meal and fasting is needed for accurate measurement, which limits their diagnostic value in rabbits because of cecotrophy. Decreased cholesterol levels in rabbits might be found in cases of liver failure, chronic malnutrition, and even pregnancy (up to 30% below the range) (Melillo, 2007). The biochemical values for rabbits Cholesterol (3.37 - 4.43mmol/L) reported in this study is slightly higher than the range (0.1-2.1) reported by medirabbits, (2012). This could be due to high level of fat in the concentrate diets as the T1 group that was fed sole concentrate has a higher level (4.43) than any other group in the study. High cholesterol is closely linked with other medical problems that means it can also happen as a result of other diseases, but the inclusion of the two vegetable leaves decreased the cholesterol level of the rabbits from (4.43) in the control to the low of (3.37) in the vegetable- based diet.

A significant difference ($P < 0.05$) was observed with the range of (0.67-1.40 mmol/L) in the triglyceride levels. The range obtained from this study fell within the normal physiological range reported by medirabbit, (2012) which is 1.4-1.76 mm0l/l. The addition of the two vegetables (cabbage and lettuce) increased the triglycerides level of the growing rabbits. This is healthy for the rabbits as it has been reported that increasing medium chain triaglycerides (MCT) levels can improve growth performance, immune, antioxidant functions and intestinal health of weaned rabbits as demonstrated by Bin *et al* (2022) where MCT was used to substitute soya bean oil. The significant difference observed from the study in the HDL and the values obtained ranged from 0.83 to 1.87 mmol/l with T1 been higher than the other groups. This may be due to the higher level of fat in the T1 diet as higher inclusion of oil has always been seen to increase serum HDL –Cholesterol levels

Alanine aminotransferase (ALT)

The increase in serum ALP has been used as a reliable indicator of cholestasis and possibly bile duct obstructions (Wulkan, 1986). The non-significant value for serum ALP were within the range of (24 to 28.33 iu/L), this fell within the normal range (12-96iu/L) reported by Melillo, (2007) and 10-96 iu/L (medirabbits, 2012). The liver enzymes AST and ALT are biomarkers used for assessing liver functions. Elevation in serum AST and ALT activity are sensitive biomarkers of liver damage (Mayer and Harvey 1998; Varga, 2013). Although higher AST levels may be found in patients diagnosed with liver damage, struggling during collection or hemolysis of the sample also raises AST levels, (Melillo, 2007). The observed values for the liver enzyme AST (25-28.67 iu/L) and ALT (20.33-28.33) in this study were lower than the range (35 to 130 iu/L) and (45 to 80 iu/L) respectively reported by Melillo (2007) but the ALP in this study (24.00 to 28.33) were within (10 to 98 iu/l) for ALP reported by medirabbits. (2012). slightly increased ALT levels are a common finding in apparently healthy rabbits. The ALT of T3 was higher than that

of T2. ALT is not increased by restraint in the rabbit, so an elevated ALT values is suggestive of tissue damage (Melillo, 2007). Several factors elevate the ALT levels; exposure to low level of lead or mycotoxins (Harcourt-Brown 2002).These results appear to indicate that the nutrients in the different treatment diets fed to the rabbits were balanced and hence supported their normal performance to maintain biochemical components of the animals. These results together with the fact that there was no mortality or health related problems recorded during the study indicate that concentrate even when given at 3% of the animals live weight supplemented with high level protein forages that are vegetables such as cabbage and lettuce can lead to optimum growth in rabbits.

Conclusion

The results obtained from this study showed that discarded cabbage and lettuce leaves in rabbit diets is non-toxic as they were observed value similarities and apparent conformities with those reported in normal, healthy and physiological conditions in rabbits and there was no mortality during the study. Therefore, concentrate feed restriction up to 3 % BWT with *ad libitum* cabbage and lettuce leaves may not elicit deleterious physiological responses**.** Animal nutritionist can conveniently use cabbage and lettuce leaves as a roughage source in growing rabbit ration

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