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Effect of Graded Levels of Essential Oil of *Zingiber officinale* on *in Vivo* Digestibility of *Pennisetum clandestinum* and Some Hemato-Biochemical Parameters of West African Dwarf Goat

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Abstract **– The study of the effect of graded levels of the essential oil of *Zingiber officinale* (ginger) on the *in vivo* digestibility of the *Pennisetum clandestinum* (Kikuyu grass) hay and some hemato-biochemical parameters of West African Dwarf goat was conducted at the University of Dschang. Nine (9) old West African Dwarf goats were used in this study. The periods of adaptation and data collection were respectively 6 and 15 days. After the adaptation, each animal received 900 and 100 g/d hay of *P. clandestinum* and concentrate respectively, associated with 0 mg essential oil of *Z. officinale* (FP + HEZo 0) for group 1 ; 100 mg essential oil of *Z. officinale* (FP + HEZo 100) for group 2 and 200 mg essential oil of *Z. officinale* (FP + HEZo 200) for group 3. The samples of 100g of each diet, of faeces and 10 ml of urine were collected for the analyses of the chemical composition and the evaluation of ingestion and digestibility. Also blood samples were obtained from jugular vein of all goats after *in vivo* digestibility test for the dosage of hemato-biochemical parameters. Results of this study showed that the ingestions of dry matter, organic matter and neutral detergent fiber were significantly ($p<0.05$) higher on the West African Dwarf goat with the diet FPc + HEZo 200. Digestibilities of these components were also higher ($p<0.05$) with the diet FPc + HEZo 200 (69.67%; 67.67% and 78.00% respectively). Ingested (3.35 g/j) and digested nitrogen (48.67%) were significantly ($p<0.05$) higher with the diet FPc + HEZo200. The values of the blood metabolites studied were not significantly ($p>0.05$) influenced by the graded levels of essential oil in diets, except the globulin and glucose. In the same way, the studied hematologic parameters were not significantly ($p>0.05$) influenced by the graded levels of essential oils of *Z. officinale* in diets. In general, the graded levels of the essential oil of *Z. officinale* in diets improved ingestion, digestibility of *P. clandestinum* and biochemical parameters of West African Dwarf goat.**

Keywords **– Hay, Digestibility, Essential Oil, Biochemical Parameters, Hematological Parameters.**

I. INTRODUCTION

Nutrition of ruminants is controlled by the microbial fermentation that occurs in the rumen. This fermentation could be improved in many ways by improving fiber digestion as well as decreasing protein degradation [1]. The handling of the ruminal ecosystem to increase the capacity of digestion of poor fodder, to reduce methane emission and nitrogen excretion by ruminants are components of fundamental objectives that seek nutritionists [2]. Digestion is a major phenomenon in the nutrition of the ruminants, the objective of the nutritionists aims of which to control the activity of the ruminal ecosystem to optimize its operation and the digestive use of fodder in order to improve the performances of production and the quality of the products [3]. Digestion in the goat leads to losses nitrogenized in the urine or milk and, with an energy loss via the production of methane, gas for purpose of greenhouse, which impacts negatively on the environment [4].

Microbial digestion in the rumen is a major phenomenon whose efficiency can be improved by selective antimicrobial agents such as, antibiotics [5]. Many food additives were developed to improve the effectiveness of the use of the nutriments by decreasing the total quantity of methane or ammonia produced. Among these additives, ionophores antibiotics such as monensin were employed successfully as food additives for decades to handle ruminal fermentations [5] so as to improve the efficiency of feeding in the system of ruminant production [6]. However, the risk of the presence of antibiotic residues in milk, meat and its effects on human health led to its prohibition in animal feeding by the European Union [7]. This prohibition caused a renewed interest for the natural substitutes which represent vegetable extracts (tanins, saponins, essential oils). Plant extracts contain secondary metabolites which showed important selective antimicrobial activities [8]-[1]. The use of essential oils (EO) in livestock nutrition has been expanded after the ban on the use of antibiotic as growth promoters, and as ionophores [9].

The EO are blends of secondary metabolites that are commonly extracted by steam distillation or solvent extraction [10]. Chemically, they are characterized as having a very diverse composition, nature and activities [11]. The effect of essential oils depends on their structure, which results from the chemical composition and the type of functional group or the aromatic molecules which compose them [3].

Several Essential Oils and their active components have strong and selective antimicrobic activities against a broad range of micro-organisms, including the bacteria, the protozoa and mushrooms [8], and can be employed to control competition between various microbial populations with the objective to improve the effectiveness of use of energy and protein of fodder in rumen [12]. Macheboeuf *et al.* [13] showed besides that there are EO amounts which allow a reduction of the proteolytic activity of the ruminal ecosystem without disturbing to a significant degree its production in volatile fatty acid (VFA). The effects of essential oils in the rumen are based on the reduction of degradation of proteins, starch and with an inhibition of degradation of amino acid due to the selective action on the micro-organisms, specifically the proteolytic, producing bacteria of ammonia, methanogens and certain pathogenic bacteria [14]; [3]. What could modulate favorably ruminal fermentations by increase in the concentration of volatile fatty acid, of the quantity of amino acids available for the needs for the animal and, by the reduction of the ammonia and methane concentration produced in the rumen [3]. However, no study was undertaken on the effect of various levels of incorporation of the essential oil of *Zingiber officinale* on ingestion, the digestive use of the hay of *Pennisetum clandestinum*, the biochemical and hematologic parameters at the West African Dwarf goat. The objective of this study is thus to evaluate the effect of various levels of incorporation of the essential oil of *Z. officinale* on ingestion, digestive use of the *P. clandestinum* hay and some biochemical and hematologic parameters at the West African Dwarf goat.

II. Materials and Methods

2.1. *Study Area*

This study was conducted at the experimental farm and Laboratory of Animal Nutrition of the University of Dschang. The geographic area is within the Sudano-Guinean zone of Central Africa (latitude 5˚ - 7˚N, longitude

8˚ - 12˚E; altitude 1400 m ASL). The annual temperature varies between 16 ˚C and 27 ˚C with a relative humidity of 40% - 97%. There are two main seasons: the rainy season (April-October) and the dry season (November-March), which is the main cropping season. The mean of the annual rainfall is about 2000 mm [15].

2.2. *Animal Material*

Nine adults and empty West African Dwarf goat of average weight 18 ± 2 kg were purchased on the market of Dschang. Their ages, determined from their dentition varied from 18 to 24 months. A month prior to the study, all animals were treated with oxytetracycline (20%), a preventive antibiotic. They were also dewormed with Ivermectin 1% (synthetic broad spectrumanthelmintic active against gastrointestinal nematodes and pulmonary adults and larvae).

2.3. *Plant Material*

Two species of plants (*Zingiber officinale* and *Pennisetum clandestinum*) were used in this study. The rhizomes of *Z. officinale* were collected in Santchou (West-Cameroun) in October 2019. These rhizomes were crushed in a fresh state for the extraction of essential oil. Fresh leaves of *P. clandestinum* were cut 45 days after planting at the experimental farm of the University of Dschang and then air dried in open shaded air at ambient temperature. A sample (100 g) was collected and analyzed and the chemical composition is presented in Table 1. The concentrated food used in this study consisted of: 50% maize, 30% wheat bran and 20% cotton seed cake. This concentrate was used as support in which was built-in essential oil to be used for the animals in order to limit evaporation.

Table 1. Chemical composition of *P. clandestinum*.

Chemical Composition	Quantity
Dry matter (%)	96
(% MS)	
Ash	15.19
Organic matter	84.80
Total nitrogenized matter	13.37
Lipids	2.09
Crude fiber	30.42
Neutral detergent fiber	82
Total carbohydrate	64.82
Acid detergent fiber	41.05
dMO	32.91

dMO: digestibility of organic matter.

2.4. *Extraction of Essential Oil*

Rhizomes of *Zingiber officinale* were crushed to a powder to enable the extraction of EO which was done in the laboratory of physiology and animal health of the faculty of agronomy and agricultural science by hydro distillation according to Wang and Waller [16]. The latter technique consists of placing the *Z. officinale* plant material in an alembic, and heating with water to 200 ˚C. Intense heat causes the explosion of oil containing saccules of *Z. officinale* to release oil which spreads in the water vapor. Oil water vapor was then channeled in a condenser and cooled to be liquefied again. At the end, oil was separated from water and was dried using anhydrous sodium sulphate.

2.5. *In Vivo Digestibility*

The nine animals were randomly divided into three groups of comparable weight. They were placed in individual cages with a device enabling separate collection of urine and faeces samples. Each group received one of the following diets:

- Group 1 (control): 100 g of concentrate + 900 g of *Pennisetum clandestinum* hay + 0 mg essential oil of *Zingiber officinale* (FPC + HEZo0).
- Group 2: 100 g of concentrate + 900 g of *Pennisetum clandestinum* hay + 100 mg essential oil of *Zingiber officinale* (FPC + HEZo100).
- Group 3: 100 g of concentrate + 900 g of *Pennisetum clandestinum* hay + 200 mg essential oil of *Zingiber officinale* (FPC + HEZo200).

The trial period of the experiment lasted 21 days including 15 for adaptation and 6 days of data collection. The aim of the adaptation period was to make it possible for animals to familiarize themselves with the digestibility cages and their new diet. During the trial period of the experiment, each animal received 500 g of the experimental diet the first day. This quantity was gradually increased until each animal received 100 g of concentrate and 900 g of *P. clandestinum* hay on the last day of adaptation. Water was available *ad libitum*. Essential oil was incorporated in the concentrate before being served to the animals. Diets was distributed twice per day at 0.5 kg at 8 am and at 0.5 kg at 3 pm). Refused *P. clandestinum* was removed daily and weighed prior to distribution of new diet. Quantity of ingested *P. clandestinum* was determined by subtracting the weight of refused quantity from the served quantity. A sample of refused diet was taken to determine the content of the dry matter. Every morning, faeces produced by each animal were weighed and urines were measured using a test-tube out of glass graduated of 500 ml. The urines produced by each animal were collected in containers in which was introduised before 10% diluted sulphuric acid according to the average volume of urine produces by each animal during the adaptation period (2.5 ml sulphuric acid for 100 ml urine) to stabilize urinary nitrogen. The volume of the urines thus collected every morning was measured using a 1000 ml graduated test-tube. 10 ml samples of urine were collected in clean bottles and preserved at 4 ˚C in a refrigerator for later analysis of nitrogen. A sample of 100 g faeces was collected and dried to a constant weight at 60 ˚C in a ventilated drying oven, prior to being crushed and preserved for letter chemical analysis. The apparent digestibility coefficient (ADC) of dry matter (DM), of organic matter (OM), of Neutral Detergent Fiber (NDF) and of nitrogen (N) was calculated respectively using the following formulas:

ADC DM (%) = (DM ingested − DM excreted) × 100/DM ingested,

ADC OM (%) = (OM ingested − OM excreted) × 100/OM ingested.

ADC NDF (%) = (NDF ingested – NDF excreted) x 100 / NDF ingested and

ADC N (%) = (N ingested − N excreted) × 100/N ingested.

2.6. *Determination of the Biochemical Parameters*

At the end of the trial of *in vivo* digestibility, blood samples were obtained from jugular vein of all goats (by puncture of the jugular vein) and introduced into labelled dry test tubes. Serum samples were collected after blood centrifugation and frozen at −20˚C until the day of analyses. They were carried out at the laboratory of Biochemistry of the University of Dschang using the commercial kits (CHRONOLAB S.A.). The experimental protocol which allowed for the determination of the serum concentration of proteinic (total protein, albumin and globulin) and energetic (glucose, triglyceride, total cholesterol, HDL) parameters in serum samples was described by the manufacturer (CHRONOLAB S.A.). Proportionings were colorimetric and the readings of absorbance were done using a spectrophotometer with the values of absorbance (λ) varying from one parameter to another.

2.7. *Proportioning of the Hematologic Parameters*

The blood taken by puncture of the jugular vein after the test of *in vivo* digestibility was introduced into heparins tubes and was used at the laboratory for the establishment of the hemogram using an automatic hematimeter to obtained the following parameters: white blood cells, lymphocytes, monocytes, granulocytes, red blood cells, haemoglobin, hematocrite, average corpuscular volume (ACV), average corpuscular concentration of haemoglobin (ACCH) and red cell distribution width (RDW-CV).

2.8. *Statistical Analysis*

Data on ingestion, *in vivo* digestibility, biochemical and hematologic parameters were subjected to one way analysis of variance (ANOVA) following General Linear Models. When differences exist between treatments, means were separated by the Waller Duncan test at 5% significance level [17].

III. RESULTS

3.1. *Ingestion of Dry Matter (DM) Organic Matter (OM) and Neutral Detergent Fiber (NDF) Inwest African Dwarf Goat According to Experimental Diets*

The incorporation of the essential oil of *Z. officinale* rhizomes significantly ($p < 0.05$) influenced ingestion of the dry matter, organic matter and neutral detergent fiber of the various diets in West African Dwarf goat (Table 2). Indeed, the ingestion of DM, OM and NDF of the FPc + HEZo200 diet were significantly ($p < 0.05$) higher than that of the diets FPc + HEZo0 and FPc + HEZo100, with the lowest values of these components obtained with the FPc + HEZo0 diet.

Table 2. Ingestion of DM, OM and NDF in West African Dwarf goat according to experimental diets.

Ingestion (g /d)		Rations		SEM	p
	FPc + HEZ$_o$0	**FPc + HEZ$_o$100**	**FPc + HEZ$_o$200**		
Dry matter	667.31^c	694.00^b	735.67^a	0.15	0.01
Organic Matter	589.66^c	612.67^b	650.00^a	0.24	0.02
Neutral detergent fiber	547.35^c	568.66^b	603.00^a	0.56	0.00

a, b, c: the mean bearing the same letter in the same line are not significantly different ($p > 0.05$) SEM: Standard error of mean; p: Probability.

3.2. *Digestibility of Dry Matter (DM), Organic Matter (OM) and Detergent Fiber (NDF) in West African Dwarf Goat According to Experimental Diets*

The incorporation of the essential oil of *Z. officinale* rhizome significantly ($P < 0.05$) improved both the digestibility of the fiber, dry and organic matter of diets in goats (Table 3). Indeed, the digestibilities of these components significantly ($P < 0.05$) increased with the level of addition of essential oil with the diet. The highest digestibilities of DM, OM and fiber were obtained with FPc + HEZ_O200 diet while lowest were obtained with FPc + HEZ_O0 diet.

Table 3. Digestibility of Dry Matter (DM), Organic Matter (OM) and neutral detergent fiber (NDF) in West African Dwarf goat according to experimental diets.

Apparent digestibility	**Rations**			**SEM**	**P**
	FPc + $HEZ_0$0	**FPc + $HEZ_0$100**	**FPc + $HEZ_0$200**		
Dry matter	53.33^c	63.67^b	69.67^a	0.74	0.01
Organic Matter	49.00^c	61.00^b	67.67^a	0.11	0.001
Neutral detergent Fiber	64.00^c	72.67^b	78.00^a	0.22	0.01

a, b, c: The mean bearing the same letter in the same line are not significantly different ($p > 0.05$) SEM: Standard error of mean; p: Probability.

3.3. *Effect of Various Levels of Incorporation of the Essential Oil of Z. officinale (HEZo) on Ingestion and Digestive use of Nitrogen of P. clandestinum Hay*

Highest, but comparable ($p > 0.05$) ingestions of nitrogen were observed with the diets FPc + HEZo100 and FPc + HEZo200 (Table 4). In the same way, quantities of nitrogen excreted in faeces of the animals receiving diets that contain the essential oil were comparable and significantly ($P < 0.05$) weaker than those excreted in faeces of animals receiving diet without essential oil. Moreover, the quantities of urinary nitrogen were comparable ($P > 0.05$) whatever the ration consumed. The quantity of nitrogen retained significantly ($P < 0.05$) increased with the level of incorporation of the essential oil of *Z. officinale* leaves. The quantity of nitrogen retained by these animals fed on diet with 200 mg essential oil was significantly ($P < 0.05$) higher than those of the animals fed on other diets. The incorporation of the graded levels of the essential oil of *Z. officinale* in the diet made it possible to increase ($P < 0.05$) ADC of nitrogen of *P. clandestinum* hay in the goat.

Table 4. Ingestion and digestive use of nitrogen in West African Dwarf goat according to experimental diets.

Nitrogenized Assessment	**Rations**			**SEM**	**P**
	FPc + HEZO0	**FPc + HEZO100**	**FPc + HEZO200**		
Ingested nitrogen (g/j)	14.00^a	14.67^a	15.67^a	0.27	0.25
Fecal nitrogen (g/j)	10.33^a	9.47^b	9.33^b	0.19	0.01
Urinary nitrogen (g/j)	2.00^a	2.33^a	2.67^a	0.15	0.29
Retained Nitrogen (g/j)	2.33^c	2.87^b	3.35^a	0.19	0.01
ADC N (%)	26.33^c	42.67^b	48.67^a	0.15	0.00

a, b, c: The mean bearing the same letter in the same line are not significantly different ($p > 0.05$) SEM: Standard error of mean; p: Probability.

3.4. *Effect of Graded Levels of the Essential Oil of Z. Officinale on Some Biochemical Parameters of West African Dwarf Goat*

The incorporation of the essential oil of *Z. officinale* in the diets influenced the serum level of some proteinic (total protein, albumin, globulin) and energetic (triglyceride, total cholesterol and HDL) parameters in West African Dwarf goat (Table 5). The incorporation of 200 mg essential oil of *Z. officinale* in the diets significantly ($P < 0.05$) increased the serum level of globulin and glucose in the goat.

Table 5. Some biochemical parameters on West African Dwarf goat according to experimental diets.

Parameters	Characteristics	Rations			SEM	p
		FPc + HEZ$_O$0	FPc + HEZo100	FPc + HEZ$_O$200		
Proteinic Parameters (g/dl)	Total proteins	3.42^{a}	4.91^{a}	6.8^{a}	0.36	0.06
	Albumin	2.4^{a}	2.67^{a}	3.00^{a}	0.34	0.49
	Globulin	1.05^{b}	2.24^{a}	3.79^{a}	0.2	0.04
Energetic parameters (mg/dl)	Glucose	54.28^{c}	55.7^{b}	62.61^{a}	0.22	0.01
	Triglycerides	20.09^{a}	25.66^{a}	27.42^{a}	0.22	0.13
	HDL	20.27^{a}	23.15^{a}	27.19^{a}	0.17	0.25

a, b, c: The mean bearing the same letter in the same line are not significantly different ($p > 0.05$) SEM: Standard error of mean; p: Probability; HDL: High density lipoprotein.

3.5. *Effect of Graded Levels of the Essential Oil of Zingiber Officinale on Some Hematologic Parameters on West African Dwarf Goat*

The variation of the hematologic parameters according to various essential oil amounts of *Z. officinale* in the diets (Table 6) showed that the incorporation of EO in the diets influenced in a variable way the hematologic parameters in the goats. Indeed, these parameters remained comparable ($p>0.05$) between the levels of incorporation of essential oil in the diets although the highest values were obtained with the FPc + HEZo200 diet.

Table 6. Variation of the some hematologic parameters according to the amount of the essential oil of *Z. officinale* on West African Dwarf goat.

Hematologic Parameters	Rations			ESM	P
	FPc + HEZ$_O$0	FPc + HEZ$_O$100	FPc + HEZ$_O$200		
WBC (10^3/µl)	27.53	26.76	23.20	0.97	0.148
Lymphocytes (10^3/µl))	25.73	25.80	22.50	1.00	0.27
Monocytes (10^3/µl)	2.10	1.90	2.20	0.19	0.84
Granulocytes (10^3/µl)	3.86	3.73	3.33	0.31	0.33
RBC (10^6/µl)	5.34	6.08	5.69	0.44	0.83
Haemoglobin (g/dl)	10.80	12.06	11.93	0.58	0.68
Hematocrite (%)	15.26	17.93	14.66	1.03	0.44

Hematologic Parameters	Rations			ESM	P
	FPc + HEZ_O0	FPc + HEZ_O100	FPc + HEZ_O200		
ACV (fl)	29.36	31.80	29.66	0.63	0.26
ACCH (g/dl)	72.50	66.00	73.00	2.11	0.41
RDW-CV (%)	16.63	16.13	16.50	0.34	0.28

SEM: Standard error of mean; p: Probability; WBC: White blood cells; RBC: Red blood cells; AC: Average corpuscular volume; ACCH: Average corpuscular concentration of haemoglobin and RDW-CV: Red cell distribution width.

IV. Discussion

The results of the effect of the essential oil of *Zingiber officinale* on food ingestion and apparent digestibility in West African Dwarf goat showed that ingestions of the dry matter (DM), the organic matter (OM) and neutral detergent fiber (NDF) were significantly improved by incorporation of essential oil in the diets whatever the level. These results are in agreements with those obtained by Kung *et al.* [18]; Incharoen and Yamauchi [19], Herawati *et al.* [20], UNO [21] with the rhizomes of zingiber meal used as additive in the food of chickens. These authors allotted this positive effect to the flavour and savour of zingiber [22]. Mekuiko *et al.* [9] also recorded similar results when they used the essential oil of *Callistemon viminalis* like additive in the dietof the West African Dwarf goat.

The apparent digestibility coefficient of the dry matter (DM), the organic matter (OM) and the neutral detergent fiber (NDF) with the FPc+HEZo200 diets were significantly higher than those reported with the other diets. This increase due to the action of principal active compounds of the essential oil of *Z. officinale* on the micro-organisms which modulate favorably ruminal fermentations and consequently improve digestibility. *Zingiber officinale* essential oil also makes it possible to increase the gastro-intestinal enzymatic secretions [23]-[4]-[9].These observations are reinforced by the results obtained by Zhao *et al.* [24] which reported that the Zingiber increases the digestion of fodder as well as nutritive absorption because of its positive effect on gastric secretion and the digestive enzymatic activities.

The effect of graded levels of the essential oil of *Z. officinale* (HEZo) on the digestive use of nitrogen of the hay of *P. clandestinum* showed that the apparent digestibility coefficient of nitrogen increased significantly with the addition of essential oil with the various diets. These results in agreement with those of Newbold *et al.* [25] and Hristov *et al.* [26].

The effects of essential oils in the rumen are based on the reduction of degradation of proteins and with an inhibition of degradation of amino acid due to the selective action of their terpenoides components on the micro-organisms, specifically the proteolytic and ammonia producing bacteria [14]-[4]. What could favorably modulate ruminals fermentations by increasing the quantity of amino acids available for the animal needs could consequently involve an increase in the digestibility of nitrogen. These results corroborate the assertion that addition of essential oil with the ration promotes an increase in the quantity of protein that goes towards the intestine thus improving the efficient use of food proteins [4]. This result suggests that the ruminal by-pass protein were increased by the addition of the essential oil, which is a beneficial effect of essential oil [25]-[27]-[4]. The values of these components in general are higher than those were obtained by Lin *et al.* [28] with a complex made up of eugenol, carvacrol and cinnamaldehyde incorporated in the proportions of 1 g/d in the diet of the sheep. This variation could be related to the difference on the level of the phytochemical composition of these oils.

Biochemical parameters studied increased with the addition of essential oil in the diets without overflowing the normative values defined by Ndoutamia and Ganda [29] although no significant difference was observed, except the average serum's glucose and globulin concentration which significantly increased with the diets FPc+HEZo+100 and FPc+HEZo+200. The high serum concentration of total protein and globuline which is the proteinic parameters of the metabolism, along with the incorporation of essential oil in the diet can be justified by the effect of terpenoid of this oil on the micro-organisms of the rumen, specifically the proteolytic and ammonia producing bacteria which promote a reduction in degradation of the proteins and with an inhibition of the degradation of amino acid that will be used in an efficient way on the level of the intestine [14]-[9]. These observations are reinforced by the results obtained with other EO containing the similar components [23]-[30]. The studied hematologic parameters remained comparable independently of the diets. These results corroborate those obtained by El-Halim *et al.* [31] which did not observe significant differences between the hematologic values of the reference group compared to the sheep having received the diets containing the essential oil of *Nigella sativa* during 6 weeks. This similarity would be due to the nature and the phytochimic composition of two oils since the action of essential oils depends primarily on their principal active components.

V. Conclusion

At the end of this study on the effect of various levels of incorporation of the essential oil of *Z. officinale* on the *in vivo* digestibility of *P. clandestinum* and some hemato-biochemical parameters on West African Dwarf goat, it arises that: the inclusion of the various essential oil levels of *Z. officinale* made it possible to obtain significantly high ingestion of DM, OM, NDF and nitrogen in this animal. This incorporation also significantly improved the digestibility of the DM, OM and nitrogen at all tested levels. Biochemical parameters studied were improved with the addition of essential oil in the diets. The graded levels of the essential oil of *Z. officinale* did not significantly influence the hematologic parameters.

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