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Comparative studies on the effects of digestive enzymes or humic acid on growth performance, some immunological and biochemical parameters of lambs

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Abstract

The objective of this work was design to investigate the effect of digestive enzymes or humic acid on body performance, some immunological and biochemical parameters of lambs. Thirty lambs aged 4 month old weighting 12 –15 kg belonged to a private farm at Sharkia Province were divided into three equal groups. 1st group healthy lambs (control group), 2nd group healthy lambs received 300mg digestive enzyme (kezyme)/kgm ration for 30 successive day, 3rd group healthy lambs received 100 mg humic acid/kgm ration for 30 successive day. At 1st and 10th day post treatment two blood samples were collected for hematobiochemical analysis. Effect of humic acid and digestive enzymes in body performance was studed .

Lambs received digestive enzyme or humic acid each alone revealed improve in weight gain, feed conversion rate beside significant increases in leukocytic count, lymphocyte, phagocytosis, phagocytic index, killing %, total proteins, albumin and gamma globulin coupled with significant decreases in neuterophils, monocytes beside insignificant increase serum of alpha , beta globulin, AST, ALT, ALP, A/G ratio, urea, creatinine, eosinophil, basophil, superoxide dismitase, catalase and non significant reduction in serum total lipid; cholesterol ,triglycerides and serum malondiahyde.

It could be concluded that digestive enzyme and humic acid induced improvement in body performance, immunological and its effect in some biochemical parameters in lambs so; it is good to use Kemzyme and humic acid during lambs fattening period

Keywords: *Digestive enzymes or humic acid, performance Blood parameter, lambs*

Introduction

Sheep and goats represent one of most important animals among animal live stock in which they have a considerable important role in many countries as providing milk, meat, skin and manure (**Haenlein and Ramirez 2017**).

Antibiotics have been used in sub therapeutic dose in animal ration to inhibit growth of intestinal pathogens and increase body weight but created antibiotic residue and development of antibiotic-resistant bacteria **(Jensen 1998)**. Growth promoters can be defined as substances which will increase growth rate and increase feed efficiency (**Sabina and Ligita 2012).**

Researchers have looked for new feed additives without harmful effect (**Ceylan *et al.* 2003).** Growth promoters are chemical and biological substances (probiotics, prebiotics, enzymes, acidifiers, antioxidants and absorption enhancers) which are added to feed to improve growth of livestock (**Zhou, *et al.* 2009).**

Enzymes used as a feed and water supplement to enhance performance and immune responses (**Cowieson 2015)**. They improve nutritive value of ration**,** improve digestibility of animal ration so improve growth (**Okorie *et al.* 2011)**. Eenzymes are mainly related to improvement in the apparent metabolically energy of diet (**Hong *et al* 2002**). Multienzymes enhance the energy utilization and improved body weight gain and feed conversion ratio **(Wyatt *et al.* 1999).**

Humic acids are a class of compounds resulting from decomposition of organic matter is natural constituents of drinking water and soil **(Vetservis, 2012)**. Its used as feed additives in animal ration to be as a part of replacement therapy for digestive disturbances as diarrhea **(EMEA 1999)**. It has antioxidant, immunostimulatory and hepatoprotective effect (**Ozturk *et al.*, 2012 and Abdel-Khalek, *et al*. 2019**)

This study was conducted to determine the effects of digestive enzymes or humic acid on body performance, immunological and biochemical parameters in lambs.

Materials and Methods

Digestive enzymes (Kemzyme®**)** it is a powder mixture of enzyme (α amylase, hemicellulose, β glucan lipase, cellulose & proteases) obtained from Kemin Comp Egypt

Humic acid (Humax®) Each kilogram contain (70% humic acid, 14.50% moisture, 3.6 % crude protein, 0.05% crude fat, 17.35% total ash and 9.0% sodium) obtained from Farmavet International Inc., Kocaeli, Turkey.

Experimental design

Thirty lambs, 4 month old, 12 – 15 kg body weight belonged to a private farm at Sharkia Province were divided into three equal groups (10 in each). 1st group clinically healthy lambs (control group), 2nd group healthy lambs received 300mg digestive enzyme (kezyme)/kgm ration for 30 successive days, 3rd group healthy lambs received 100 mg humic acid/ kgm ration for 30 successive days

Body weight

All lambs were individually weighed at begining of the experiment and at 1st days post treatment, amount of feed used was recorded. Where body weight gain, feed conversion rate were calculated.

Blood samples

At 1st and 10th days post treatment two Blood samples were collected from all lambs via Jugular vein puncture from each lamb,

1st sample was tacken on tube contain anticoagulant to estimation leukogram (**Jain 2000**), Phagocytic activity, index and Killing % **(Kawahara *et al.* 1991**)

2nd sample was collected in test tube to obtain clear serum for estimation total protein (**Doumas, *et al.* 1981**) Serum protein fractions were performed using cellulose acetate electropheresis test (**Henry, et al.1974**), aspartate aminotransferase and alanine aminotransferase **(Reitman and Frankel 1957),** alkaline phosphate (**John 1982**)**,** triglyceride **(Wholerfeld 1974),** urea **(Patton and Crouch 1977**), creatinine **(Husdan and Rapoport 1968**), Catalase **(Aebi 1984),** Super oxide dismutase **(Nishikimi *et al* 1972)** malondialdhyde **(Ohkawa *et al* 1979).**

Statistical analysis obtained data were analyzed by t test (**Pertri and Watson 1999)**

Results and Discussion

Dietary addition of humic acid and digestive enzymes each alone revealed a significant increas in weight gain and improvement in feed conversion rate in lambs (table1). Elevation in body weight and improvement in feed conversion rate may be due to antimicrobial effect of humic acid on rumen bacteria (**Ivan *et al.* 2004**) beside humic acid can form a protective film on the mucus epithelium of gastrointestinal tract against infections and toxins, thus improved utilization of nutrients in animal feed (**Islam, *et al.* 2005).** Humic acid in diets improve growth and feed conversion efficiency (**Mellor and Lück-**

städt, 2011). Same improvement in weight gain and feed conversion rate was observed by **El-Zaiat, *et al.* (2018)** in kids received humic acid. Elevation in body weight and improvement in feed conversion rate may be due to enzymes improve digestibility of nutrient (**Bedford 2000)** Same increase in body weight was observed in pigs received enzyme (**Bedford and Schulze 2009)**.

In the current study, as showed in table (2) humic acid and digestive enzymes each alone induced significant increases in leukocytic count, lymphocyte, phagocytosis%, phagocytic index, killing %, coupled with significant decreases in neuterophils, monocytes and insignificant increas in eosinophils and basophils at the 1st day post administration coupled with insignificant effect at 10th day post administration. Same changes in blood picture was reported by **Griban *et al.* (1991)** who stated that the use of humic acid induced improved immune system of calves. Also, **Klocking, (1994) and (Chang -Hua *et al.* 2003)** reported that humic acid has immunostimulant and improved phagocytic activity of leukocytes due to activation of neutrophils**.** Changes in leukogram, phagocytosis, killing % may be due to immunostimulatory effects of humic acid **(Joone, *et al.* 2003).** Our results were supported by previous studies **Habibian, *et al.* (2010)** stated that humic acid impoved leukocytic count, phagocytosis, phagocytic index, and killing %. Our data are fit with that obtainned by **Diah, *et al.* (2016**) who concluded that the addition of humic acid to a western rats diet resulted significant increase in leukocytic count, lymphocyte, phagocytic activity associated with significant decreases in neuterophils, monocytes and insignificant increase eosinophils and basophils. Similar findings were reported by **Agazzi, *et al.* (2007)** who mentioned that dietary humates increased leukocytic count, phagocytosis, phagocytic index, and killing % in newborn kids.Same change in total and diferenial leukocytic count, phagocytosis, phagocytic index, killing % were observed by **Rivero *et al* (2012)** in growing lambs received exogenous enzyme. Our recorded data were similar to previous report (**Sarat, *et al.* 2012**) they found that daily addition of exogenous enzymes in rabbit ration for 30 days elicited significant increase leucocytic count, lymphocytes, neutrophiles, phagocytosis, phagocytic index, killing %.

In the present study, table (3) showed that, healthy lambs received humic acid or digestive enzymes resulted in a significant increase in total protein, albumin , globulin and gamma globuin all over the experimental period post administration beside non significant increase serum alpha and beta globulin. Increase in serum total proteins, albumin and globulin may be due to increase in digestion, absorption and increase protein anabolism (**Bedford and Schulze 2000).** Same results were recorded by **Wang, et al. (2008) and El-Zaiat, *et al.* (2018)** in pigs and in goats received humic acid respectively. Humic acid increased total protein and globulin **(Vetvicka, *et al.* 2010** and **Trckova *et al.*, 2005).** Multi enzymes induced elevation in total protein albumin and globulin in sheep (**Kina, *et al.* 2004**). Cattle received digestive enzymes showed significant increase in total protein, albumin and globulin (**Ayad, *et al.* 2013)**

Analysis of serum as presented in table (3) revealed that insignificant reduction in total lipid; cholesterol and triglycerides all over the experimental period post feeding lambs on ration contain humic acid and digestive enzymes each alone. Humates have inhibiting effect on pathogenic bacteria secrete inflammatory agents increasing the protein synthesis in liver, and so increase in serum total proteins (**Klasing and Austic 1984**). In an earlier study, **Nurten, *et al.* (2010)** reported that rams received humic acid showed insignificant decrease in total cholesterol. Humate had a reductive effect on total lipid, cholesterol and triglyceride level **(Galip, *et al.* 2010)**. Our data are fit with that obtained by **Tunc and Yoruk (2017)** who recorded that humate additive in ration of newly borne calves revealed insignificant effect in serum triglycerides and total cholesterol. Humate additives decreased the level of cholesterol **(El-Zaiat, *et al.* 2018)**.

Dairy cattle received enzymes showed reduction in cholesterol and triglyceride **(Ayad *et al.* 2013**). Same result was recorded by **Dailidavičienė, *et al.* (2018)** who stated that exogenous enzyme induced reduction in total lipid and cholesterol in cattle.

The obtained results in table (4) revealed that humic acid and digestive enzymes each alone induced insignificant increase in serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphate, urea and creatinine in lambs all over the experimental period post administration**.** Our results were supported by previous studies **Nurten, *et al*. (2010)** reported that rams received humic acid revealed insignificant increase in aspartate aminotransferase, alanine aminotransferase, alkaline phosphate, urea and creatinine. In the same line **Vetvicka, *et al.* (2010)** and **Tunc and Yoruk (2017)** stated that humate induced non significant increase in serum aspartate amino-transferase, alanine aminotransferase, alkaline phosphate,, urea and creatinine. Enzymes induced non significant increase in serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphate, urea and creatinine (**Kina, *et al.* 2004**). Dairy cow received digestive enzyme revealed insignificant elevation in serum liver enzymes, urea and creatinine **(Dean, *et al.* 2013).**

In the current work as presented in table (5), it has been found that, humic acid and digestive enzymes each alone induced non significant increase in serum superoxide dismitase, catalase coupled with insignificant decrease in malanodialdhyde in lambs. Same result was recorded by **Tarasova, *et al.* (2015)** and **Kamel, *et al.* (2015)** stated that humic acid has a powerful antioxidant activity and could protect the cells from oxidative stress and damage by increasing the glutathione reductase, catalase activity and decrease the malondialdehyde lipid peroxidase. Also, **Tunc and Yoruk (2017)** stated that humate additive in ration newly borne calves revealed non significant effect in blood serum superoxide dismitase and increased malanodialdhyde levels, . In the same line **Ginis, *et al.* (2012**) concluded that the addition of multienzymes to a rats diet resulted in an insignificant decrease in malanodialdhyde coupled with insignificant elevation in serum superoxide dismitase and catalase.

It could be concluded that the humic acid and digestive enzymes each alone induced improvement in body performance, immunological and its effect in some biochemical parameters in lambs so; it is good to use Kemzyme and humic acid during lambs fattening period

Table (1): Effect of enzymes and humic acid on body weight gain (WG), feed consumption (gm/lamb) (F.C.), feed conversion rate (F.C.R.) of lambs (n=5)

Period parameters	GP 1	GP 2	GP 3
Intial body weight	14.83±0.43	14.79±0.21	14.80±0.51
Final body weight	22.18±0.32	24.04±0.28	24.26±0.20
WG	7.35±0.21	9.25±0.17	9.46±0.19
F.C.	22.5	22.5	22.5
F.C.R	3.06	2.43	2.38

Table (2): Effect of enzymes and humic acid on leukogram, phagocytosis, phagocytic index and Kiling % of lambs (n=5)

Period parameters		1st day			10th day		
		GP 1	GP 2	GP 3	GP 1	GP 2	GP 3
leukocytic count		10.12± 0.26	11.17± 0.32*	11.87± 0.42*	10.32± 0.33	10.51± 0.42	11.56± 0.34
Differ-ential count	Lymphocyt	3.65± 0.22	3.98± 0.12*	3.89± 0.18*	3.69± 0.21	3.85± 0.19	3.80± 0.18
	neuterophil	2.82± 0.18	2.30± 0.14*	2.22± 0.17*	2.79± 0.19	2.70± 0.23	2.75± 0.15
	Eosinophil	1.40± 0.32	1.61± 0.19	1.65± 0.21	1.42± 0.18	1.59± 0.21	1.58± 0.21
	Basophil	1.18± 0.22	1.31± 0.17	1.29± 0.25	1.11± 0.21	1.23± 0.29	1.17± 0.12
	Monocyte	1.07± 0.11	1.97± 0.13*	2.02± 0.25*	1.14± 0.21	1.21± 0.18	1.26± 0.18
Phagocytiosis		69.05± 0.88	73.43± 0.79*	73.86± 0.85*	69.21± 0.63	70.89± 0.79	70.26± 0.85
phagocytic index		6.30± 0.49	7.89± 0.38*	7.58± 0.42*	6.48± 0.84	6.95± 0.28	6.75± 0.62
Killing %		38.84± 1.70	41.93± 0.89*	41.27± 0.68*	38.38± 0.98	39.39± 0.78	39.05± 0.81

*Significant at $P \leq 0.05$

Table (3): Effect of enzymes or humic acid on protein profile (g/dl) and lipid profile (mg/dl)of lambs (n=5)

Period parameters			1st day			10th day		
			GP 1	GP 2	GP 3	GP 1	GP 2	GP 3
Protein profile (gm/dl)	T. protein		7.43± 0.28	8.94± 0.24*	8.77± 0.28*	7.51± 0.23	8.49± 0.21*	8.20± 0.12*
	Albumin		4.07± 0.39	4.79± 0.30*	4.93± 0.28*	4.10± 0.21	4.59± 0.18*	4.38± 0.27*
	Globulin	alpha	1.04± 0.14	1.37± 0.18	1.21± 0.18	1.15± 0.12	1.19± 0.17	1.16± 0.13
		beta	1.12± 0.18	1.33± 0.19	1.22± 0.19	1.12± 0.15	1.15± 0.19	1.14± 0.16
		gamma	1.20± 0.16	1.55± 0.19*	1.41± 0.13*	1.13± 0.14	1.56± 0.12*	1.52± 0.10*
		total	3.36± 0.23	4.15± 0.31*	3.84± 0.17*	3.41± 0.29	3.90± 0.19*	3.82± 0.21*
	A/G Ratio		1.20± 0.12	1.15± 0.10	1.20± 0.13	1.20± 0.11	1.18± 0.11	1.03± 0.13
Lipid profile	T lipid (mg/dl)		278.29± 9.45	269.08± 10.95	272.43± 7.89	279.06± 6.77	276.54± 5.98	277.43± 5.64
	Cholsterol (mg/dl)		74.53± 3.32	69.64± 2.89	70.72± 4.59	76.17± 6.54	72.21± 8.48	72.70± 5.49
	Triglycerid (mg/dl)		27.46± 10.54	22.28± 9.87	20.05± 7.84	26.38± 8.62	25.1± 9.43	26.55± 6.48

Table (4): Effect of enzymes and humic acid on liver enzymes and kidney function of lambs. (n=5)

Period parameters		1st day			10th day		
		GP 1	GP 2	GP 3	GP 1	GP 2	GP 3
Liver enzymes (U/L)	AST	42.51± 0.56	42.93± 0.33	42.81± 0.29	42.62± 0.84	42.70± 0.23	42.68± 0.34
	ALT	29.41± 0.44	29.85± 0.31	29.66± 0.22	29.39± 0.79	29.53± 0.29	29.42± 0.51
	ALP	32.16± 0.62	32.69± 0.42	32.42± 0.28	32.27± 0.75	32.32± 0.21	32.30± 0.17
Kidney function (mg/dl)	Urea	25.48± 0.42	25.78± 0.73	25.49± 0.21	25.51± 0.33	25.44± 0.19	25.38± 0.28
	Creatinine	1.63± 0.06	1.75± 0.03	1.72± 0.09	1.66± 0.09	1.73± 0.08	1.69± 0.06

Table (5): Effect of enzymes and humic acid on oxidative stress of lambs (n=5)

Period parameters	1st day			10th day		
	GP 1	GP 2	GP 3	GP 1	GP 2	GP 3
CAT U/ml	74.27± 0.83	76.06± 0.98	75.12± 0.64	74.69± 0.48	74.87± 0.73	74.22± 0.58
SOD U/ml	95.37± 0.89	96.17± 0.69	95.85± 0.48	95.59± 0.66	96.05± 0.82	95.62± 0.79
MDA nmol/ml	79.38± 0.82	77.78± 0.59	78.96± 0.87	79.21± 0.67	78.59± 0.84	78.32± 0.71

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