

THE EFFECT OF YEAST (*SACCHAROMYCES CEREVISIAE*), GARLIC (*ALLIUM SATIVUM*) AND THEIR COMBINATION AS FEED ADDITIVES IN FINISHING DIETS ON THE PERFORMANCE, RUMINAL FERMENTATION, AND IMMUNE STATUS OF LAMBS

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SUMMARY

Four groups of male lambs (seven animals/ group) were used to study the effect of natural (garlic powder; *Allium sativum*) and biological (dry yeast; *Saccharomyces cerevisiae*) additives and their combination in finishing diets as compared to control diet on feed utilization and animal performance. Animals were 8 months of age and $35.8\text{kg} \pm 0.41$ as average body weight. Animals were fed a basal diet including concentrate feed mixture (CFM) at level of 70% of total requirement and berseem hay (BH) was offered *ad lib*. The experimental diets were: 1) a basal diet without additive (control), (C); 2) a basal diet supplemented with 6g dry yeast (2.44×10^{11} cfu/g)/head/day, (Y); 3) a basal diet supplemented with 40g garlic powder/head/day, (G), and 4) a basal diet supplemented with 3g dry yeast plus 20g garlic powder/head/day, (YG). The results revealed that all feed additive treatments showed higher ($P < 0.05$) digestibility values of DM, OM, CP, CF and NFE, than non-additive diet (C). The highest ($P < 0.05$) values were observed for animals fed G diet; however, C group showed the lowest ($P < 0.05$) digestibility values. The G diet showed the highest ($P < 0.05$) value of TDN% (73.56%) and C showed the lowest one (69.20%). However, the DCP% was not affected ($P < 0.05$) by additives and its values ranged between 11.81 and 12.27%. Animals fed enriched diets (Y, G and YG) showed higher ADG (180, 184 and 186 g/d, respectively) compared to control group (160g/d). Additives have no significant effect on feed intake either in the form of CFM or the roughage and consequently the total feed intake. All feed additives and their combination significantly ($P < 0.05$) enhanced, with the same extent, the feed efficiency indicators of the enriched diets compared with control one. Yeast/garlic combined addition revealed the highest daily profit percentage relative to control (42%) followed by garlic (34.0%) and then yeast alone (31%) treatments. Energy utilization was significantly different ($P < 0.05$) between the test groups where, the G group showed the highest values, but C group was the lowest values. When the combined additive (YG) was supplemented, N balance exhibited 15.2% increase above the control group. The concentration of blood immunoglobulins (IgA) and IgG differed ($P < 0.05$) among groups being their concentration were enhanced by the respective additives. It could be concluded that using feed additive such as dry yeast (6 gm/h/d) or garlic powder (40 gm/h/d) or their combination (3gm plus 20 gm, respectively) in finishing diets of lamb tended to increase digestibility coefficients for most of nutrients, increasing nutritive value as TDN and appeared to increase the daily gain as well as enhanced the immune status of animals.

Keywords: Yeast, garlic, lambs, performance, *in vitro*, fermentation and blood.

INTRODUCTION

The ruminant livestock industry plays a major role in the production of both of meat and milk as a key source of protein for human consumption. Sheep worldwide are mostly owned by poor rural families who lack modern management skills, and thus have poor feeding and housing practices with insufficient adoption of technologies which are important to improve productivity. Various dietary additives are widely used in ruminant diets modulate rumen metabolism, which ultimately improves nutrient use and animal performance. Enhancing feed quality and utilization by using certain feed additives may be considered a partial vertical solution to the problem of negative feed balance of total digestible nutrients

(TDN) and digestible crude protein (DCP) which stated for a long period in the animal production sector of Egypt. There is need to save about 3.4 million tons of TDN over the total actual amount produced (9.6 million tons) to cover that required (13.0 million tons) as reported by Alnaimy *et al.* (2017).

Many workers applied chemical substances (antibiotics and hormones) as growth promoters in animal feeding to enhance the growth rate and to provide significant economic income. Using Herbs, spices, have received greater attention as potential alternatives to antibiotic growth promotants, since they are considered as natural products (Abd El-Latif *et al.*, 2019). There are numerous investigations that focused on utilization of natural plant as feed additives in animals (Frankic *et al.*, 2009). The addition of herbal additives to animals feed can also help stimulate the immune response (Khosravi *et al.*, 2010) and improve digestibility of the feedstock, thereby enhancing quality of locally available feed source which in turn, helps to increase the production of sheep in our country. Among the well-known herbal additives include garlic which is small herbal plant of 30 to 50cm height and belongs to the Amaryllidaceae family. Garlic has many medicinal properties and is known to improve the immune system. Garlic (*Allium sativum*) has anti-microbial, anti-oxidant, and anti-hypertensive properties and has been used as a flavor in the animal nutrition industry (Rivlin, 2001 and Sivam, 2001).

There is no study focused on the impacts of yeast and garlic powder together on the rumen fermentation patterns and nutrient digestibility. It was hypothesized that the combination of both products may have an additive effect on stimulating fermentation and digestion of plant cells in the rumen. So the main research objective of this study is to examine the effect of yeast or garlic powder and combination of both as additives on lamb performance. The specific objectives of this study were to evaluate growth response of finishing lambs as well as economic efficiency; to evaluate the apparent digestibility of nutrients and rumen fermentation in lambs fed diets supplemented with yeast and/or garlic powder and to assess the impacts of feed additives on the immune status of animals.

MATERIALS AND METHODS

The farm experiment and the lab work were carried out at Animal Production Department, Faculty of Agriculture, Menoufia University, and the part of the fermentation study was accomplished at Maryout Research Station, Desert Research Center, Ministry of Agriculture.

Growth trial:

A complete randomized block design was followed in a growth trial for twenty-four male lambs which were divided into four groups (seven lambs/ group). Each group was housed in three pens and distributed as follows: 2, 2, and 3 lambs/pen.

Animals were 8 months of age with an average body weight of 35.8kg (\pm 0.41 SE). Animals were weighted weekly before morning feeding. Animals were fed a basal diet (control) including concentrate feed mixture (CFM) at level of 70% of total requirement of growth as recommended by NRC (1985) and berseem hay (BH) was offered *ad lib*. The control ration was offered to the first group without additives, while the other three experimental groups received the control ration supplemented with dry yeast and/or garlic powder. The investigational diets were: 1) a basal diet without additive (control), (C); 2) a basal diet supplemented with 6g dry yeast (2.44×10^{11} cfu/g)/head/day, (Y); 3) a basal diet supplemented with 40g garlic powder/head/day, (G), and 4) a basal diet supplemented with 3g dry yeast plus 20g garlic powder/head/day, (YG). The concentrate feed mixture was formulated as follows: yellow corn 50%, cotton seed meal 25.0%, wheat bran 22.0%, limestone 1.6%, common salt 1.0%, mineral and vitamin mixture 0.4%. The amount of CFM was offered daily in two portions at 09:00 AM and 17:00 PM; however, water was available for animals all times throughout the experiment.

Immunological blood parameters for lambs:

Blood samples were collected in two tubes before feeding via the jugular vein from each lamb. The first tube to separate the blood plasma so it contains ethylene tetra acetic acid (EDTA) to prevent blood clotting to the white blood cell (WBC's) count (Kolmer *et al.*, 1951). The other tube for separating blood serum so it was without anti-coagulant and centrifuged 2h after collection at 3500 rpm for 20 minutes, then analyzed for immunoglobulin A (IgA) and immunoglobulin G (IgG) using enzyme-linked immunosorbent assay method (Thomas, 1998).

Economic indicators:

Market prices in 2019 were used to calculate the economic indicators expressed in Egyptian pounds (L.E.). The prices were assigned as follows; Berseem hay L.E. 2900/ton; concentrate feed mixture L.E. 4500/ton; dry yeast L.E. 22/ kg; garlic powder L.E. 35/ kg and live body weight L.E. 60/ kg.

Digestibility, nitrogen balance, and water metabolism trials:

Twelve adult rams (50.41kg±0.61) were placed in individual metabolic cages (1.6 m x 0.53 m) and offered the same previous four rations (3 rams/ rations) for two weeks as an adaptation period followed by a week-long collection period. Water was offered twice daily, and water intake was recorded. Daily excreted feces from each animal were collected. Exactly 20% of the weight-based samples were taken and dried at 60 °C for 72 hours. Urine was collected daily in plastic jars, acidified (using 100 ml of 4N H₂SO₄), measured and 10% of the volume was sampled for nitrogen determination. Feed and fecal samples were ground through a 1 mm sieve on a Wiley mill grinder and sub samples were taken for each animal for subsequent analysis. Feed and fecal samples were analyzed for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash. Urine samples were analyzed for nitrogen (N) content accordance with the AOAC (2005). During the collection period, free drinking water intake was recorded. Daily water excretion through feces and urine were estimated. Data of water intake and excretion were related to the live body mass (BW^{0.82}) of the animals as advised by Macfarlane and Howard (1970).

Rumen fermentation trials:

Four adult rumen-cannulated Barki rams with an average body weight of 47.9.0 ±1.7 kg were arranged in Latin square design for four periods (15days each) to study the effect of experimental diets on the deferential protozoal count and other fermentation criteria. Ruminal contents were sampled at zero, 3, 6 and 12h post-morning feeding to record the pH and determine concentration of NH₃-N, and volatile fatty acid (VFA). Ruminal pH was immediately measured using a digital pH meter (WPA CD70). Samples of rumen fluid were analyzed for ammonia-nitrogen (NH₃-N) according to Preston (1995). Total volatile fatty acids (TVFAs) were determined using steam distillation method described in Warner (1964).

Apart from the collected sample at zero time were immediately flittered through one layer of gauze, then fixed and stained with 4 times the volume of salt solution of green-methyl formalin as described by Ogimoto and Imai (1981), then stored in a dark place until examination. Subsequent identification of genera and protozoa species was followed as described in Dehority (1993).

The *in vitro* gas production technique was used to estimate *in vitro* degradation of both DM and OM (IVDMD & IVOMD), as well as methane production for experimental regimes (as total mixed rations). Ruminal content (50:50 v/v) collected via cannula was squeezed through four-layered cheesecloth and the filtrate liquid was incubated in a water bath at 39 °C saturated with CO₂ until further inoculation. The incubation medium was prepared according to Menke *et al.* (1979) description. Each 200 mg DM sample was incubated in 100-mL glass serum bottles where 30 mL of the incubation medium was added. The samples were incubated in triplicate and the cumulated gas production was monitored 3, 6, 12, 24, and 48h post incubation. Three bottles containing rumen juice and artificial saliva without sample were used as blank to correct for gas production values released from rumen contents.

Data on gas production were adapted for to the following model from France *et al.* (2000): $A = b \times [1 - e^{-k(t-L)}]$, Where: A is the volume of gas production at time t; b is the asymptotic gas production (based on mL/200 mg DM); k is the rate of gas production per hour from the slowly fermentable feed fraction b; and L is the discrete lag time prior to gas production.

The partitioning factor (PF) was calculated as the ratio between the true digested DM (mg) to the volume of gas (mL in 24 h).

IVDMD and IVOMD were determined after terminal of each incubation time by recovery of the undigested fraction. The true degradability of DM and OM (TDDM & TDOM) was determined at 24h after reflux residual content with a 50 mL neutral detergent solution for 3h at 105°C. After recording the final gas volume at 24h of incubation, 4 ml NaOH (10 M) was injected in each bottle to measure methane volume as described by Demeyer *et al.* (1988).

The microbial protein (MCP) was calculated in accordance with Czerkawski (1986), where 19.3 g microbial nitrogen produced per kg TDOM.

Calculations and statistical analysis:

Growth energy (GE) and digestible energy (DE) for the tested rations were calculated by equations reported by Nehring and Haenlien (1973) as follows:

$$GE \text{ (M cal/kg DM)} = 5.72 \text{ CP} + 9.50 \text{ EE} + 4.79 \text{ CF} + 4.03 \text{ NFE}$$

$$DE \text{ (M cal /kg DM)} = 5.72 \text{ DCP} + 9.05 \text{ DEE} + 4.8 \text{ DCF} + 4.06 \text{ DNFE}$$

Metabolizable energy (ME) was calculated as $DE \times 0.82$ (NRC, 1985). The net energy for maintenance (NEm) or growth (NEg) was computed as outlined by Rattray *et al.* (1973).

$$NEm = 0.79 \text{ ME} - 0.4 ; NEg = 0.58 \text{ ME} - 0.52$$

Data of growth and digestibility trials were analyzed using Statistical Analytical System (SAS, 2002), Version, 9.3.1, according to the General Linear Model as a completely randomized design with animals as block. The model of statistics was the following: $Y_{ij} = \mu + T_i + e_{ij}$

Where: Y_{ij} = the observation; μ = Overall mean; T_i = the fixed effect of the treatments; e_{ij} = Random error component assumed to be normally distributed.

The statistical model associated with a Latin square design for the fermentation trials is:

$Y_{ijk} = \mu + \alpha_i + T_j + \beta_k + \varepsilon_{ijk}$; Where μ is the baseline mean, α_i is the block effect associated with row i , β_k is the block effect associated with column k , T_j is the j^{th} treatment effect, and ε_{ijk} is a random error.

The Duncan multiple range test was performed (Duncan, 1955) to detect significant differences among means.

RESULTS AND DISCUSSION**Chemical composition of feeds and experimental feed additives:**

The proximate composition of the bakery's yeast biomass (*Saccharomyces cerevisiae*) presented in Table (1) is characterized by high levels of protein (45.74%) and ash (6.79%). The CF content was low being value 2.51%. Total carbohydrates (45.72%) represent nearly half of the biomass. The present results agree with those recently reported by Elaref *et al.* (2020), which were 91.83, 82.58, 46.13, 3.85 and 39.24% respectively for DM, OM, CP, EE and NFE. The present values of CP, CF, EE% and Ash were higher than those found by Ibrahim and El Naggat (2018) who reported 40.82, 1.10, 1.33 and 2.98% for the same items in dry yeast, but our findings were lower in case of DM (87.05%), OM (97.02%) and NFE (53.77%). Compared with the present results of dry yeast contents, Abu El-Kassim *et al.* (2018) reported a lower DM, OM, CP and NFE values (92.58, 86.78, 14.10 and 50.22%, respectively) but cited higher values of CF, EE and Ash (19.74, 2.71 and 5.80%, respectively).

Table (1): Chemical composition of experimental feeds, additives and rations.

Item	Experimental feed ¹		Feed additive		Experimental ration ²			
	CFM	BH	Dry yeast	Garlic powder	C	Y	G	YG
Moisture, %	13.03	13.98	13.08	5.99	13.30	13.30	13.13	13.22
DM, %	86.97	86.02	86.92	94.01	86.70	86.70	86.87	86.78
Nutrients % on DM basis								
CP	13.05	12.53	45.74	20.44	12.90	13.04	13.13	13.09
CF	11.21	28.40	2.51	1.66	16.03	16.06	15.65	15.96
EE	3.86	3.53	1.75	1.38	3.77	3.76	3.71	3.73
NFE	63.95	37.84	43.21	72.59	56.62	56.48	57.18	56.67
TC ³	75.16	66.24	45.72	74.25	72.66	72.54	72.83	72.63
Ash	7.93	17.70	6.79	3.93	10.67	10.65	10.33	10.55

¹CFM: Concentrate feed mixture; BH: Berseem hay.

²C: control diet CFM plus BH, Y: control diet enriched with yeast (6gm/h/d), G: control diet enriched with garlic powder (40gm/h/d), YG: control diet enriched with yeast (3gm/h/d) plus garlic powder (20gm/h/d).

³TC: Total carbohydrates = OM - (CP + EE).

The chemical composition of garlic powder (Local market product) is shown in Table (1). The results are similar to the earlier results of Otunola *et al.* (2010), where moisture, CP, EE, CF, total carbohydrates and ash contents in garlic sample were 4.55, 15.33, 0.72, 2.10, 73.22 and 4.08% respectively on dry basis (95.45%). Petropoulos, *et al.* (2018) studied 14 Greek garlic genotypes, and they detected significant differences in nutrients contents. The range values on DM basis were calculated to be 31.67- 42.64% DM, 3.59-5.73% Ash, 10.83-22.85% CP, 0.28-1.10% Fat, 70.93-84.50% carbohydrate, 94.27-96.41% OM and 3821-3901 Kcal/kg DM as an energy content. Garlic is relatively high in CP (20.44%), which is close to that declared by Sahli *et al.* (2018) which found a value of 22.9% and 18.8%, respectively. However, Abu El-Kassim *et al.* (2018) reported a lower CP value (14.10%). Garlic contained appreciable amounts of carbohydrates and protein and these results emphasize that it can be classified as carbohydrate and protein-rich spice (Abayomi *et al.*, 2018). Furthermore, the high CP content of garlic was due to the presence of active metabolites such as allicin, ajoene and capsaicin as reported earlier by Dashak *et al.* (2001). The herein values from this study were higher than those reported for moisture and CP at 4.88 and 17.35% respectively by Nwinuka *et al.* (2005) and similar to the values of 73.03, 0.68, 4.06% reported for carbohydrate, ether extract and ash contents, respectively by the same authors. The result is in disagreement with the reports from Mariam and Devi (2016) who stated that, garlic contains 3.91% moisture, 19.75% CP, 0.49% EE, 1.73% CF, 66.36% carbohydrate and 3.39% ash on dry matter basis. Observed differences in chemical composition compared to literature may be related to genetic varieties and possibly to substrate analyzed mainly for cell wall. Furthermore, Petropoulos *et al.*, (2018), indicated that apart from the genotype, both of growing conditions and cultivating practices also have a significant impact on the feeding value of garlic bulbs. In addition, plant density, fertilizer application rate and soil type have been reported to significantly affect the protein content of garlic bulbs (Diriba-Shiferaw *et al.*, 2014).

Animal performance and economic indicators:

The ADG values revealed significant ($P<0.05$) differences across experimental groups (Table 2), where animal groups fed enrichment diets (Y, G and YG) showed higher values compared to control group (160 g/d) but on the same time the treated groups were comparable between each other. Relative to control group, the feed additives improved ADG by 12.5, 15.0 and 16.25% for Y, G and YG, respectively. Higher ADG in animals fed yeast supplemented diets (Y and YG) can be attributed to improved propiono-genesis process via yeast (Kawas *et al.*, 2007). Malekkhahi *et al.* (2015) reported no effect of yeast culture supplementation on ADG or FCR in growing lambs. Hassan and Mohammed (2014) concluded that the addition of *S. cerevisiae* to the high concentrate diet improved the digestibility of CP and CF as well as ADG (143.7 g/d) in Awassi male lambs. Numerous studies (Garg *et al.*, 2009; Milewski, 2009) have reported an increasing gain due to the addition of *S. cerevisiae* to sheep diet. Ahmed and Salah (2002) estimated a higher increase (13.8 and 30.2%) as a result of the addition of *S. cerevisiae* to sheep at a rate of 4 and 8 g/day, respectively, compared to the control diet. Maamouri *et al.* (2014) recorded 145 g/day and 223 g/day as the daily weight gain for respective lambs of (C) group and (Y) one. Payandeh and Kafilzadeh (2007) found that finishing lambs received diet supplemented with *S. cerevisiae* had a significantly higher ADG (209 vs. 177 g day⁻¹) but without positive effect on feed conversion ratio. These results are in harmony with those obtained on West African Dwarf goats fed diets supplanted with garlic powder (Ikyume *et al.*, 2017) or sheep supplemented with yeast (Zeid *et al.*, 2011). However, the current results contrast with that those of Tatara *et al.* (2008) who reported that garlic did not have a significantly effect on the growth rate although an improvement in growth was observed. Additionally, Bampidis *et al.* (2005) reported that weight gain was not significantly affected by dietary garlic pulp and husk supplementation in growing lambs compared to control group. Garlic powder supplementation in Ikyume *et al.* (2017) study had no significant effect on the feed conversion ratio (FCR) of West WAD goats. This result is consistent with that found by Strickland *et al.* (2009) where the inclusion of raw garlic in the diet of Merino lambs aged 6 months reduced the FCR.

Data concerning feed intake based on DM basis (Table 2), indicates that, the feed additive have no effect on feed intake either in the form of CFM or the roughage and consequently the total feed intake. Feed intake (g/head/d) expressed as TDNI was affected by the experimental feed additives, where animal's group fed G diet showed the highest value of TDNI but the C group showed the lowest value and the other two groups (Y and YG) were similar. On the other hand, DCPI expressed as g/head/d (Table 2) was not affected ($P<0.05$) by treatments although G group showed higher value compared with the other three groups. The lack of effect of Yeast and/or garlic powder on DMI in this study may be attributed to the high proportion of concentrates (high energy intake) in the diet. These results agreed with Hassan and Mohammed (2014) and Malekkhahi *et al.* (2015) who found no significant impact on DM intake as a result of *S. cerevisiae* supplementation. However, on contrary to current results, a positive influence of SC on DM intake in growing animals was observed by Lascano *et al.* (2009).

Table (2): Changes in body weight, consumption criteria and economic indicators for lambs fed with enriched diets.

Item	Experimental diet				SEM	P value
	C	Y	G	YG		
Body weight						
IBW, kg	35.7	36.5	35.2	36.0	1.26	0.779
FBW, kg	44.5	46.4	45.3	46.2	0.88	0.610
ADG, g	160 ^b	180 ^a	184 ^a	186 ^a	3.23	<0.001
Advantage, %	-	12.5	15.0	16.25	-	-
Feed & nutrients intake (g DM/head/d)						
CFM	1042	1045	1075	1035	10.69	0.207
B. Hay	405	415	402	410	12.17	0.884
Total DMI	1447	1460	1477	1445	17.82	0.569
TDN	1001 ^b	1050 ^{ab}	1086 ^a	1050 ^{ab}	20.24	0.051
DCP	171	177	181	177	3.78	0.292
Feed utilization efficiency (g/g)						
DM/Gain	9.04 ^a	8.11 ^b	8.03 ^b	7.77 ^b	0.19	<0.001
TDN/Gain	6.26 ^a	5.83 ^{ab}	5.90 ^{ab}	5.65 ^b	0.16	0.039
DCP/Gain	1.07 ^a	0.98 ^b	0.98 ^b	0.95 ^b	0.03	0.028
Economic indicators ¹						
Price of daily gain, LE	9.60	10.80	11.04	11.16	-	-
Daily CFM cost, LE	5.39	5.54	6.96	6.13	-	-
Daily hay cost, LE	1.37	1.40	1.36	1.38	-	-
Total daily feed cost, LE	6.76	6.94	8.32	7.51	-	-
Daily Profit, LE	2.84	3.86	2.72	3.65	-	-
Relative daily profit, %	100	136	96	129	-	-
Improvement, %	0.0	36.0	-4.0	29.0	-	-

¹Price of year 2019; CFM 4500 LE/T, BH 2900 LE/T, Yeast, 22 LE/Kg, Garlic 35 LE/Kg, LBW 60 LE/ Kg.

Economic indicators were calculated based on as fed basis including feed additives.

a and b means at the same raw with different superscript letters are significantly ($P < 0.05$) different.

Data of feed utilization efficiency expressed as DM/Gain, TDN/Gain or DCP/Gain are present in Table (2). The finding provides evidence that all feed additives and their combination significantly ($P < 0.05$) enhanced, with same extent, the previous mentioned feed efficiency indicators of the enriched diets compared with control one. The best feed efficiency obtained by YG additive may be attributed to the beneficial effects of yeast plus garlic. Where yeast provided stimulator factors and essential nutrients specially protein, energy, minerals and vitamins that better utilized by sheep (Zaki, 2016). These factors and essential nutrients resulted in some change in the digestive function that led to increasing the availability and utilization of nutrients in the rumen and could have a significant impact on the feed utilization and growth rate. Moreover, garlic has improved the use of energy and nitrogen from the diet. Briefly, it could be concluded that, yeast and/or garlic powder improved DM/Gain by 10.29, 11.17 and 14.05%, TDN/Gain by 6.87, 5.75 and 9.74% and DCP/Gain by 8.41, 8.41 and 11.21%. This result confirms that a low growth rate lead to a high ratio of feed to live weight gain. These results are consistent with those found by Maamouri *et al.* (2014) for yeast and Ghosh *et al.* (2010) for garlic powder. However, Hassan and Mohammed (2014) found that addition of *S. cerevisiae* (5 g/ head/d) in lamb diets had no effect on FCR value, also Zhong *et al.* (2019) found that lambs fed basal diet without or with 50g garlic powder per kg diet for 84d resulted in no significant change in feed conversion ratio.

The economic indicators were calculated for the animal groups fed the experimental finishing diets under the present study (Table 2). Its logic matter to find supplemented diets revealed higher daily feed cost compared to control one. All treatment groups revealed higher daily gain and consequently resulted in higher price. From these results, it could be concluded that adding yeast and yeast plus garlic powder to rations of lambs were more effective in increasing the daily profit percentage relative to control, being values 36 and 29%, respectively. However, garlic alone revealed a negative value mainly because of its high price so the use of garlic additive in finishing diet of growing lambs is restricted by a low price case.

Immunological blood indicators:

The data presented in Table (3) summarized the effect of the experimental finishing diets on immunological blood indicators in lambs. An increase in the ratio of neutrophils to lymphocytes (N/L ratio) as an immune parameter proposed as a marker of chronic stressful situations in farm animals (Trevisi and Bertoni, 2009). The N/L ratio in the present study (Table 3) showed progressively higher values with group C (0.24), which is indicative of stress. A higher N/L ratio may also indicate a health issue for the animal, reflecting a weakened immune system and often as a result, an unhealthy animal (Hyun-Sun *et al.*, 2009). The lower N/L ratio observed in the respective feed additive groups probably reflects an identical humoral immune response in lambs. Data in Table (3) revealed that immunoglobulins (IgA) and IgG differed ($P < 0.05$) among groups being their concentration were enhanced by the respective additives. These results were consistent with those obtained by El-Shereef (2019).

Table (3): Immunological blood indicators in sheep fed yeast and/or garlic supplementation.

Item	Experimental diet				SEM	P Value
	C	Y	G	YG		
Neutrophils (N), ($\times 10^3/\mu\text{l}$)	17.63 ^a	6.50 ^b	6.93 ^b	7.10 ^b	1.22	<0.001
Lymphocytes (L), ($\times 10^3/\mu\text{l}$)	72.85 ^b	87.10 ^a	79.25 ^{ab}	89.70 ^a	4.00	0.004
N/L ratio	0.24	0.07	0.09	0.08	-	-
IgA, (IU/l)	5.33 ^b	8.33 ^a	8.10 ^a	8.67 ^a	0.69	0.032
IgG, (IU/l)	7.43 ^c	12.90 ^b	12.33 ^b	14.67 ^a	0.94	0.003

SEM: standard error of the mean, P value: probability value

a, b and c means at the same raw with different superscript letters are significantly ($P < 0.05$) different.

Energy and nitrogen utilization:

Data of gross energy (GE), digestible energy (DE), metabolizable energy (ME) and net energy for maintenance (NEM) and for growth (NEg) of lambs are presented in Table (4). It could be noticed that the GE was not significantly ($P > 0.05$) different among groups, being values ranged between 5.99 and 6.14 Mcal/d. It might be due to that feed intake was not different among groups. However, other criteria of energy utilization (DE, ME, NEM and NEg Mcal/d) were significantly ($P < 0.05$) different across the experimental groups, where, the G group showed the highest values but C group was the lowest values, however both Y and YG groups showed comparable values. The same trend was observed for NEg as a ratio of GE and ME. These findings may explain the enhanced effect of the tested additives on the basal diets and was in accordance with finding of (El-Meccawi *et al.*, 2009) who stated that the energy balance of small ruminants is dependent on the quality of their diets. The TVFAs are the final products of rumen microbial fermentation and represent the major supply of ME for ruminants (Van Soest, 1982). Therefore, using garlic as a feed additive may be responsible for the improvement of energy production and carbohydrate metabolism in animal received diets supplemented with garlic. The present result of NEg was in accordance with that found by Klevenhusen *et al.* (2011) who found that the concentrate supplemented with 4 g diallyl di-sulphide (an important component of garlic oil) increased ($P = 0.07$) body energy retention to be 4.06 MJ/d as compared with un-supplemented animals (3.48 MJ/d).

The impacts of Y, G or YG additives on N intake, fecal N, urinary N and N balance are shown in Table (4). Animals fed diet supplemented with G showed slight increase ($P < 0.05$) in N intake (NI) and lower N voided via feces (FN), however, both Y and YG groups showed comparable ($P < 0.05$) values and were in between G and C groups. As DMI was similar among the experimental groups so significant differences observed in NI was not attributed to DMI but mainly to additives nitrogen intake where it was 0.44, 1.31 and 0.88 g N/d for Y, G YG groups, respectively as compared to non-additive group. No significant ($P < 0.05$) effects due to supplement of Y, G or their combination were observed on the urinary nitrogen (UN), total voided nitrogen (TVN) or its relative percentage to NI (UN/NI% and TVN/NI%) as compared to the control group. A lower ($P < 0.05$) nitrogen loss in the feces of the supplemented groups especially the focus of G group, but not observed in UN, emphasis that both of G and Y were more effective for N digestion than the absorption pathways. When the combined additives (YG) were supplemented, N balance exhibited 15.2% increase above the control group, whereas Y or G supplementation had similar values and located between Y and YG groups.

In accordance, Sallam *et al.* (2014) reported that microbial feed additives brought about less excretion of urinary and fecal nitrogen, which led to improvement in nitrogen balance. Cole *et al.* (1992) showed that lambs fed YG had higher N retention than the control which confirms our findings. The higher retention of N in group Y may be explained by the optimal ruminal $\text{NH}_3\text{-N}$ concentration that appears to

result from increased incorporation of N into microbial protein as a consequence of stimulated microbial activity (Malekkhahi *et al.*, 2015). The results of N balance in this study contrast with that of Mungoi *et al.* (2012) who reported no effect of supplementing yeast on N balance in lambs. In earlier work, Amagase (2006) found antioxidant effects for the bioactive components of Garlic could play a role in improving the use of N in sheep fed hay supplemented with garlic leaf.

Table (4): Use of energy and nitrogen in lambs fed finishing diets enriched with yeast and/or garlic powder.

Item*	Experimental diet				SEM	P value
	C	Y	G	YG		
Energy utilization:						
GE, M cal/d	5.99	6.05	6.14	5.99	0.07	0.439
DE, Mcal/d	3.74 ^b	3.92 ^{ab}	4.03 ^a	3.91 ^{ab}	0.08	0.099
ME, Mcal/d	3.07 ^b	3.22 ^{ab}	3.31 ^a	3.21 ^{ab}	0.07	0.096
NEm, Mcal/d	2.38 ^b	2.50 ^{ab}	2.57 ^a	2.49 ^{ab}	0.05	0.097
NEg, Mcal/d	1.26 ^b	1.35 ^{ab}	1.40 ^a	1.34 ^{ab}	0.04	0.105
NEg/GE%	20.93 ^b	22.23 ^{ab}	22.80 ^a	22.34 ^{ab}	0.59	0.158
NEg/ME%	40.87 ^b	41.80 ^{ab}	42.21 ^a	41.73 ^{ab}	0.39	0.111
N utilization:						
Total NI (TNI), g/head/d	29.87 ^b	30.47 ^{ab}	31.02 ^a	30.25 ^{ab}	0.36	0.174
Fecal N (FN), g/head/d	7.84 ^a	7.44 ^{ab}	6.68 ^b	7.00 ^{ab}	0.34	0.100
FN/NI %	26.25 ^a	24.42 ^{ab}	21.53 ^b	23.14 ^b	1.07	0.023
N absorbed (NA), g/head/d	22.03 ^c	23.03 ^{bc}	24.34 ^a	23.25 ^{ab}	0.41	0.003
NA/ NI %	73.75 ^b	75.58 ^{ab}	78.47 ^a	76.86 ^a	1.07	0.022
Urine N (UN), g/head/d	15.57	15.76	17.08	15.81	0.56	0.223
UN/ NI %	52.13	51.72	55.06	52.26	1.66	0.497
TVN*, g	23.41	23.2	23.76	22.81	0.48	0.570
TVN/ NI %	78.37	76.14	76.60	75.40	1.02	0.237
N retained (NR), g/head/d	6.46 ^b	7.27 ^{ab}	7.26 ^{ab}	7.44 ^a	0.29	0.094
NR/TNI, %	21.63	23.86	23.40	24.60	1.02	0.215
NR/NA, %	29.32	31.57	29.83	32.00	1.56	0.614
NR, mg/ kg BW	128.9	146.0	144.7	148.6	7.14	0.214

*GE: gross energy; DE: digestible energy; ME: metabolizable energy; NEm: net energy for maintenance; NEg: net energy for growth; *TVN: Total voided N; SEM: standard error of the mean; P value: probability value a, b and c means at the same row with different superscript letters are significantly ($P < 0.05$) different.

Digestibility and feeding values of diets:

Table (5) presents nutrients digestibility and feeding values of the experimental diets. All feed additive treatments resulted in higher ($P < 0.05$) digestibility values of DM, OM, CP, CF and NFE, but not in the C diet. In general, the highest ($P < 0.05$) values were observed in animals fed G diet. However, C group showed the lowest ($P < 0.05$) digestibility values for the same items. The digestibility of the EE was not affected ($P < 0.05$) by the experimental additives. This result may be due to the digestibility of fat is not affected by the presence of yeast in the gastrointestinal tract, since yeast do not hydrolyze bile acids, and fat emulsion in mixed micelles (El-Hennawy *et al.*, 1994). The observed increment in digestibility coefficient of major nutrients of enriched feed additive diets may be attributed to its high metabolizable energy content compared to their content of control diet (Kewan *et al.*, 2019). Otherwise, garlic powder could alter the microbial population profile, reducing the activity of *Prevotella spp* which is mainly responsible for protein degradation and amino acids deamination leading to improved protein digestion and metabolism (El-Shereef, 2019).

These results are consistent with those obtained by Zhong *et al.* (2019) which found that the digestibility of DM ($p = 0.019$) and CP ($p = 0.007$) increased by garlic powder supplementation (5% or 50g/ kg DM feed) however, lipid digestibility was not affected by the same supplementation. The values were 64.21, 74.38, 72.28% DM, CP, EE for garlic vs 60.29, 68.27, 69.97% for control. Also, the present results agree with those obtained by El-Shereef (2019) who noticed that the addition of garlic powder (2% of DMI) considerably enhanced the apparent digestibility of DM, OM, CF and CP compared to control ration being values were 59.6 vs 55.9% DMD; 56.8 vs 53.7% OM; 60.9 vs 52.5% CF; 70.6 vs 65.1% CP for garlic powder treatment vs control ration, respectively.

In contrast, Ikyume *et al.* (2017) found that including 0.5% garlic powder inclusion in the diet of West African Dwarf goats significantly ($p < 0.05$) reduced the digestibility of CP compared to the control group being values were 68.70 vs 75.78%, respectively. However, digestibility of DM, OM, CF, and EE was not affected ($p < 0.05$) by the same level of garlic powder compared to un-supplemented group. The authors suggested that garlic powder inhibits the digestibility of protein, which could be good for the animals as the protein is protected for use in the small intestine. The improvement in nutrients digestibility by yeast supplementation is compatible with the findings of Malekhhahi *et al.* (2015) who found that yeast supplementation increased the digestibility of CP and NDF. Higher increases in digestibility were observed in Awassi lambs fed a high concentrate diet supplemented by *S. cerevisiae* (Hassan and Mohammed, 2014).

Table (5): Nutrients digestibility and feeding values of diets enriched with yeast and/or garlic.

Items	Experimental diets				SEM ¹	P value ²
	C	Y	G	YG		
Nutrients digestibility (%):						
DM	72.30 ^b	75.03 ^{ab}	76.70 ^a	76.07 ^a	1.29	0.09
OM	73.53 ^b	76.37 ^{ab}	77.98 ^a	77.17 ^{ab}	1.309	0.105
CP	73.69 ^b	75.63 ^{ab}	78.47 ^a	76.89 ^a	1.07	0.022
CF	61.34 ^b	66.99 ^{ab}	67.41 ^a	66.54 ^{ab}	2.069	0.147
EE	74.72	79.40	78.14	78.11	4.65	0.905
NFE	76.87 ^b	80.00 ^{ab}	80.73 ^a	80.15 ^{ab}	1.31	0.185
Feeding values (%):						
TDN ³	69.20 ^b	71.96 ^{ab}	73.56 ^a	72.66 ^{ab}	1.32	0.124
DCP ⁴	11.81	12.14	12.26	12.27	0.19	0.315
NR ⁵	4.86	4.93	5.00	4.92	0.10	0.829
NQI ⁶	9.51 ^b	9.86 ^{ab}	10.30 ^a	10.06 ^a	0.17	0.019

¹Standard error of the means; ²Probability value; ³Total digestible nutrients; ⁴Digestible crude protein

⁵Nutritive ratio = (TDN-DCP)/DCP; ⁶Nutritive quality index = (CP %) × (DMD %) /100.

^{a,b}Means in the same row with different superscript letters are significantly ($P < 0.05$) different.

The enhancement of nutrients digestibility reflected on the nutritive value expressed as TDN% (Table 5), so that the same trend was observed where G diet showed the highest value (73.56%) but C showed the lowest one (69.20%). However, the percentage of DCP was unaffected by additives and its values varied between 11.81 and 12.27%.

The nutritive ratio (NR) showed insignificant ($P < 0.05$) differences mainly due to insignificant DCP%. The nutritive quality index (NQI) indicated that, feed additives enhanced the quality of the basal diet may be owing to significance in DM digestibility.

Calculating the improvement of TDN% achieved by additives in relative to control diet (Table 5) recoded that garlic addition was superior followed by combination of yeast and garlic and then yeast in the last (6.30, 5.00, and 3.99%, respectively). However, DCP% improved by 2.79, 3.81, 3.90% as a result of adding yeast, garlic and their half combination to the control diet. These results may be explained through the increase in favorable nitrogen source for rumen microbes beside the higher available carbohydrates which may lead to more microbial fermentation so that it reduced the dietary energy sources escaping from ruminal degradation. The present results are similar to that of Bueno *et al.* (2013) and Zeid *et al.* (2011).

Water utilization:

Water metabolism criteria are presented in Table (6). Data demonstrates that, the experimental feed additives had no effect ($p < 0.05$) on combined feed water, free water intake as related to metabolic body weight ($g/kgW^{0.82}$) or as related to dry matter intake ($g/gDMI$), and also excreted water in feces or urine. However, total water excretion expressed as $g/kgW^{0.82}$ or $g/gDMI$ was recorded to be the highest ($P < 0.05$) in control group and the lowest in G group. Both of two groups Y and YG showed comparable values and were in between C and G groups.

Animal groups fed diet included garlic powder (G and YG) showed higher insensible water loss (IWL) expressed as $g/kgW^{0.82}$ or as relative to TWI, TDNI, and DCPI. On the other hand, yeast group (Y) showed the lowest values of IWL or $gIWL/kgTDNI$.

The respective feed additives increased metabolic water intake as compared with non-additive diet but it did not reach to be significant, although mean values of combined metabolic water intake are mainly related to TDNI of each diet (Kewan *et al.*, 2017). Higher values of total water loss recorded for C group may be resulted as a consequence of higher water turnover rate and/or digesta flow (Araújo *et al.*, 2010). However, the higher ($P < 0.05$) insensible water loss observed in the YG group may be attributed to the inclusion of garlic powder which may cause increasing of heat increment that result from diet fermentation which may lead to increase water needed for body cooling system (Kewan *et al.*, 2017), however yeast group (Y) showed lower insensible water loss as the yeast may have anti-oxidative stress effects for animals (Hyun-Sun *et al.*, 2009).

Table (6): Water utilization in rams fed diets enriched with yeast and/ or garlic powder.

Item	Experimental diet				SEM	P value
	C	Y	G	YG		
Metabolic body weight, kgW ^{0.82}	24.87	24.87	24.92	24.86	0.52	0.999
Feed combined water, g/kgW ^{0.82}	8.96	9.05	8.99	8.98	0.21	0.993
Free water intake (FWI):						
g/ kgW ^{0.82}	157.7	141.8	139.6	157.7	9.65	0.381
g/ g DMI	2.69	2.39	2.34	2.67	0.13	0.122
Metabolic water, g/kgW ^{0.75}	24.29	25.45	26.45	25.42	0.76	0.356
Total water intake TWI, g/kgW ^{0.82}	191.0	176.3	175.0	192.1	10.18	0.524
Fecal water (FW):						
g/ kgW ^{0.82}	18.07	17.22	15.47	17.76	1.47	0.612
% of TWI	9.46	9.77	8.84	9.25	0.68	0.806
Urine water (UW):						
g/ kgW ^{0.82}	89.35	81.15	70.57	70.61	6.59	0.144
% of TWI	46.78 ^a	46.03 ^{ab}	40.33 ^{ab}	36.76 ^b	2.78	0.088
Total water excretion (TWE):						
g/ kgW ^{0.82}	107.4 ^a	98.4 ^{ab}	86.0 ^b	88.4 ^{ab}	7.38	0.167
% of TWI	56.23 ^a	55.81 ^{ab}	49.14 ^{ab}	46.02 ^b	2.96	0.085
g/ g DMI	1.83 ^a	1.64 ^{ab}	1.45 ^b	1.50 ^{ab}	0.11	0.068
Insensible water loss (IWL):						
g/kgW ^{0.82}	83.60 ^{ab}	77.90 ^b	89.00 ^{ab}	103.7 ^a	7.52	0.161
% of TWI	43.77 ^b	44.19 ^{ab}	50.86 ^{ab}	53.98 ^a	2.97	0.105
g/kg TDNI	2.07 ^{ab}	1.85 ^b	2.04 ^{ab}	2.36 ^a	0.17	0.218
g/g DCPI	12.15 ^{ab}	10.94 ^b	12.15 ^{ab}	13.98 ^a	0.95	0.175
g/g NR	330.4	275.7	307.6	335.4	29.64	0.479

SEM: standard error of the mean, P value: probability value

a, and b means at the same raw with different superscript letters are significantly ($P < 0.05$) different.

***In vitro* rumen fermentation parameters:**

Rumen pH, NH₃-N and TVFA:

In vitro rumen pH, NH₃-N, and TVFA concentrations at 0, 3, 6, and 12 post-feeding rams on control, Y, G and YG supplemented diets are given in Table (7). The garlic and control diets were similar ($P < 0.001$) in pH at zero time and also the same finding was observed for Y and YG diets. It can be observed that, pH value was higher at zero than other all incubation times or in other words, it almost declined with progressing of time from zero up to 12h for all the experimental diets with significant differences at all tested diets. Gradual decreasing of rumen pH with progressing time may be due to higher concentrate otherwise higher organic acids resulted from fermentation caused by feed additives may explain the gradually significant decreasing of rumen pH against the time detected in the current study. The present results contrast with those reported by Sahli *et al.* (2018) who found no changes in the *in vitro* fermentation of the rumen by including garlic powder. Also, the pH of rumen liquor was not affected by garlic treatment in sheep (Kongmun *et al.*, 2010 and Abu El-Kassim *et al.*, 2018) or dairy goats (Kholif *et al.*, 2012). On the other hand, Yang, *et al.* (2004) and Gaafar, *et al.* (2009) found that adding yeast led to an increase in ruminal pH by decreasing the ruminal lactate concentrations through increased activity of lactate fermenting bacteria (*Selenomonas ruminantium* and *Megasphaera elsdenii*) in the rumen.

Ammonia-nitrogen concentration (Table 7) was not affected ($p > 0.05$) by the experimental diets at zero time of feeding. Diets included garlic powder (G and YG) showed higher ($P < 0.001$) $\text{NH}_3\text{-N}$ concentration at early hours (3 and 6h) as compared to the other experimental diets but Y diet was the highest at the late hour (12) post-feeding. The CP content of Y, G and YG were higher than C diets owing to feed additives, so the results herein showed that supplemented diets produce higher ($p < 0.001$) $\text{NH}_3\text{-N}$ as compared with C diet. This may be explained by the possible difference in the degradability of CP in the rumen between supplemented and un-supplemented diets. The increased concentration of $\text{NH}_3\text{-N}$ suggests that yeast and garlic have increased the ruminal degradable protein and hence the ability of produce higher levels of microbial protein. Carbohydrates are the most important source of energy for the uptake of $\text{NH}_3\text{-N}$ by microorganisms; therefore, the rate of carbohydrate fermentation was highly related to the rate of rumen protein degradation to $\text{NH}_3\text{-N}$ and then production of microbial protein (Van Soest, 1982). Previous studies showed that concentration of ammonia-N decreased significantly ($P < 0.05$) in animals fed garlic (Abu EL-Kassim *et al.*, 2018) or yeast (Lascano and Heinrichs, 2009) as compared to that fed control diet. This decline may be attributed to the increased incorporation of ammonia in microbial protein (Chaucheyras and Fonty, 2001), and the stimulation of microbial activity (Lascano and Heinrichs, 2009), or it can be a direct effect of yeast on the reduction of CP degradation (Eweedah, *et al.*, 2005).

Table (7): The effect of yeast and/or garlic feed additives on *in vitro* rumen fermentation.

Incubation hour	Experimental diet				SEM	P value
	C	Y	G	YG		
Rumen liquor pH						
0	6.63 ^a	6.56 ^b	6.63 ^a	6.55 ^b	<0.01	<0.001
3h	5.87 ^a	5.78 ^c	5.80 ^b	5.81 ^b	<0.01	<0.001
6h	5.65 ^a	5.64 ^a	5.63 ^b	5.63 ^b	<0.01	0.02
12h	5.48 ^a	5.46 ^b	5.45 ^b	5.46 ^b	<0.01	0.019
$\text{NH}_3\text{-N}$, mg/dL						
0	9.80	9.80	11.55	10.15	0.55	0.16
3h	19.63 ^c	21.25 ^{bc}	34.23 ^a	22.75 ^b	0.55	<0.001
6h	24.08 ^b	24.07 ^b	26.63 ^b	33.55 ^a	0.80	<0.001
12h	23.93 ^b	26.30 ^a	16.30 ^c	22.53 ^b	0.67	<0.001
TVFA, meq/dL						
0	3.28	3.50	3.50	3.49	0.07	0.18
3h	5.50 ^b	5.05 ^b	5.53 ^b	6.28 ^a	0.16	0.005
6h	6.28 ^c	7.50 ^a	7.28 ^a	6.78 ^b	0.13	0.001
12h	9.05 ^a	8.03 ^b	8.28 ^b	8.06 ^b	0.16	0.008

SEM: standard error of the mean, P value: probability value

a, b and c means at the same raw with different superscript letters are significantly ($P < 0.05$) different.

Our results regarding ruminal pH and $\text{NH}_3\text{-N}$ disagreed with those obtained by Putnam, *et al.* (1997), where they reported no significant effect of adding yeast on the concentration of ammonia-N or the pH of the rumen fluid. This disagreement may be attributed to differences in the level of addition and/or different SC strains used. Newbold *et al.* (1995) stated that certain yeast strains are effective while others are not.

Higher values of rumen ammonia concentration at 6h for YG and at 12h in Y group may be attributed to an increase in proteolysis and protein deamination by micro-organisms and increase the ruminal non ammonia nitrogen pools resulted after addition of *S. cerevisiae* living cells (Galip, 2006). Higher value of rumen ammonia in G group at 3h was in consistence with that found in lactating cows fed garlic oil (Yang *et al.*, 2007) however Ikyume *et al.* (2017), observed reduced $\text{NH}_3\text{-N}$ concentration during fermentation as a result of garlic supplementation.

The total VFA concentration (Table 7) was not affected ($P > 0.05$) by the experimental diets at zero hour post-feeding. It was noticed that the experimental additives have main effect within 6h post feeding, where combined YG increased ($p < 0.01$) the TVFA concentration at 3h as compared to the other experimental diets. However, separate Y or G supplementation increased TVFA at 6h as compared to both of the other two groups (C and YG). The control diet showed the highest ($P < 0.01$) TVFA concentration at 12h as compared to the other supplemented diets.

The previous data concerning high TVAs at 6hr for Y group matches well to those reported for sheep by Komonna (2007). They reported that the total VFA was higher in supplemented groups with YC compared to the control group. In contrast, the work of Ismaiel *et al.* (2010) on sheep and Gado *et al.* (1998) on goats revealed insignificant differences in total VFA due to yeast culture supplementation. High TVFA at 6h for G group was in accordance with that reported by Zhong *et al.* (2019) who found that garlic powder supplementation increased total VFA in dairy goats as well as in sheep. However, Ikyume *et al.* (2017) did not observe significant differences due to garlic supplementing.

DM and OM degradability:

As apparent digestibility is not enough to evaluate the nutritive value of ruminant feeds, therefore it is necessary to determine the ruminal kinetics of digesting dietary nutrients. *In vitro* degradation data for DM and OM are presented in Table (8). Respective additive showed significant effect at all incubation times except at 12h. For DM, the intercept value (*a*) for the different treatments representing dry mater degraded (DMD) from soluble fraction ranged from 17.50 to 20.19 and it was significantly different ($P < 0.05$) among treatments. Where Y diet showed the highest value and G had the lowest one. In addition, all kinetic constants; dry mater degraded from the insoluble fraction (*b*), the potential extent of DMD (*a+b*), the degradation rate constant for the insoluble fraction (*c*); and also the effective degradability were significantly different among treatments ($P < 0.05$). It seems that G had the highest values followed by YG, Y and then C group.

The effective degradability of DM and OM for the experimental diets is given in Table (8). Data were calculated using rumen outflow rates of 2, 4 and 8% h^{-1} . There were significant differences ($p < 0.01$) among diets where G diet displayed the highest values as compared to other diets.

Table (8): The effect of yeast and/or garlic feed additives on *in vitro* degradability of DM and OM

Item	Experimental diet				SE M	Experimental diet				SEM
	C	Y	G	YG		C	Y	G	YG	
In Vitro DM degradability (%)					In Vitro OM degradability%					
0 _h	15.08 ^a	14.72 ^a	12.59 ^b	12.46 ^b	0.31	2.19	2.55	2.29	2.40	0.20
3 _h	36.57 ^b	36.22 ^b	39.47 ^a	38.04 ^{ab}	0.85	19.60 ^a	13.35 ^b	5.92 ^c	17.92 ^a	0.77
6 _h	43.53 ^{ab}	44.88 ^{ab}	48.87 ^a	42.54 ^b	1.79	31.61 ^c	35.81 ^b	34.47 ^b	40.63 ^a	0.86
12 _h	46.03	46.16	47.16	45.88	0.42	41.20 ^c	50.07 ^a	47.99 ^b	49.55 ^{ab}	0.52
24 _h	55.28 ^d	60.39 ^c	65.5 ^a	63.26 ^b	0.26	52.66 ^c	59.13 ^a	56.61 ^b	57.15 ^{ab}	0.74
48 _h	66.09 ^b	74.27 ^a	74.94 ^a	75.61 ^a	0.59	78.67 ^a	83.39 ^b	83.06 ^b	78.67 ^c	0.41
DM kinetics					OM kinetics					
A	18.73 ^{ab}	20.19 ^a	17.50 ^b	18.74 ^{ab}	0.51	7.12 ^a	3.24 ^b	1.10 ^c	6.00 ^a	0.36
B	43.05 ^b	52.79 ^a	54.01 ^a	56.33 ^a	1.12	77.01 ^b	80.16 ^b	83.98 ^a	78.15 ^b	1.02
a+b	61.78 ^b	72.98 ^a	71.51 ^a	75.07 ^a	1.34	84.14	83.40	85.08	84.15	0.97
C	0.117 ^a	0.074 ^b	0.114 ^a	0.073 ^b	0.01	0.047 ^b	0.066 ^a	0.059 ^a	0.065 ^a	>0.01
ED _{0.02}	55.48 ^b	61.71 ^a	62.91 ^a	62.97 ^a	0.39	77.01 ^b	80.16 ^b	83.10 ^a	78.15 ^b	1.02
ED _{0.04}	50.80 ^c	54.42 ^b	56.78 ^a	55.15 ^b	0.27	77.01 ^b	80.16 ^b	83.98 ^a	78.15 ^b	1.03
ED _{0.08}	44.29 ^b	45.54 ^b	48.53 ^a	45.65 ^b	0.60	77.01 ^b	80.16 ^b	83.98 ^a	78.15 ^b	1.03
True DMD _{24h}					True OMD _{24h}					
%	65.34 ^d	83.68 ^b	85.33 ^a	73.30 ^c	0.32	58.98 ^d	79.30 ^a	72.99 ^b	66.34 ^c	0.95
Improve ¹ %	18.20 ^d	38.56 ^a	30.27 ^b	19.03 ^c	0.05	11.99 ^d	34.11 ^a	28.94 ^b	16.07 ^c	0.25
Improve ² %	-	28.08	26.55	15.25	-	-	34.45	23.75	12.47	-

SEM: standard error of the mean, P value: probability value. a, b and c are degradation constants.

¹Calculated based on apparent DMD_{24h}; ²Calculated based on true DMD of control diet.

a, b and c means at the same raw with different superscript letters are significantly ($P < 0.05$) different.

True DM degradability (Table 8) was exclusively higher by G additive then Y and YG in the second category. However, the highest improvement based on apparent DMD at 24h was revealed by yeast followed by garlic powder and then YG combination. Ryan and Gray, 1989) showed that the rate of substrate fermentation increase as a result for multiplication of bacterial numbers by *S. cerevisiae* supplementation. Hadjipanayiotou *et al.* (1997) claimed that the use of *S. cerevisiae* did not affect the digestibility of nutrient, whereas Plata *et al.* (1994) found positive *in vivo* or *in situ* responses. Garlic powder supplemented treatment (16 mg) increased *in vitro* true digestibility (IVTD) as compared to the control ($P < 0.01$). However, Yang *et al.* (2007) observed that garlic supplementation did not affect the total digestibility's of DM, OM, fiber and starch, while ruminal DM and OM digestibility was increased (Kongmun *et al.*, 2010). Sahli, *et al.* (2018) demonstrated an increase ($P < 0.001$) in *in vitro* gas production with the addition of 32 and 64 mg garlic powder. They added that, TOMD was similar for all

the doses (0, 4, 8, 16, 32 mg) except for 64 mg, where a small but significant ($P < 0.001$) increase was observed (77.7%).

Ikyume *et al.* (2018) found that garlic inclusion had no ($P > 0.05$) influence on the measured *in vitro* digestibility kinetics. Where, *IDMD* was not statistically ($P > 0.05$) significant, garlic powder 0.5% group had numerically higher value (68.11%) while the control had the least value of 65.23%. The non-significantly *in vitro* organic matter digestibility (OMD) ranged between 68.47% and 84.32%. The highest percentage OMD was observed in the garlic powder 0.5% group while the control group had the least value of 68.47%. Favorable ruminal digestion responses to yeast culture feeding in sheep include an increase DM and OM degradation (Kamel *et al.*, 2004). The present results are within that range reported for roughage and concentrate by Mabjeesh *et al.* (2000) where the *IVDMD* of roughages ranged from 47 to 61%, the highest for grass hay and lowest for clover hay. The value of *IVDMD* for grains varied from 63 to 92%, the highest value recorded for corn. CP supplements also showed a wide range of *IVDMD* values, 55 to 91%, the lowest for cottonseed meal and the highest for fish meal. The *IVDMD* of whole cottonseed was low compared to other feedstuffs, averaging 38%.

All *in vitro* OM degradability (Table 8) at different incubation times and its kinetic values otherwise true OM degradability as well as its improvement based on either apparent OMD_{24h} of the same diet or true OMD for control diet were parallel to the same criteria of DMD as found by Guney *et al.* (2016). It is well accepted that the significant differences observed in OMD among the experiment diets mainly resulted from feed additive effects on the same basal diet. It means that yeast, garlic powder and its combination showed higher OM fermentation along with the different period of fermentation. So, its modifications effects are still unknown, but we can expect from the other data of the present results that yeast addition mainly affect through stimulate the fibrolytic bacteria (Chaucheyras *et al.*, 2019) or garlic supplements inhibit Archaea, which produce methane in the rumen (Kamel *et al.*, 2004). Khattab *et al.* (2010) showed that dried yeast and garlic recorded higher ($P < 0.05$) values of *IVDMD* and *IVOMD* than control. The improvement of *IVDMD* and *IVOMD* with combinations may be attributed to one or more of the following reasons; 1) available in essential ingredients such as vitamins, enzymes and essential amino and fatty acids for microflora from yeast (El-Ashry *et al.*, 2001), 2) improvement in the flora environment for better digestibility with yeast (Campanile *et al.*, 2008), 3) the medicinal oils of garlic (Khater *et al.*, 2009). It could be concluded that the present feed additive treatments were the most effective treatment in increasing dry matter and organic matter disappearances from nutritional point of view.

Rumen protozoa and total gas and methane yield:

The values of ruminal ciliate protozoa count as affected by the experimental treatments are illustrated in Table (9). A significant ($P < 0.05$) difference was observed for different differential species (*Entodinium sp.*, *Epidenium sp.*, *Diplodinium sp.*) except *Polyplastron sp.* and total count due to experimental treatments at zero-time of feeding. The present results indicated that the highest density was recorded for *Entodinium spp* which is ferment cellulose and protein while the lowest densities recorded for *Diplodinium spp* and *Polyolastron spp* which is ferment cellulose, especially that *Polyolastron spp* can digest 50% of cellulose in the rumen (Hungate, 1966). The high count of *Entodinium spp* matched with lower ruminal pH in the experimental groups as it has tolerance for lower pH (Aziz *et al.*, 2018) and can be explained that *Entodinium spp.* is responsible for the use of lactic acid formed in the rumen (Khaled and Baraka, 2011) and direct feed microbial that produce lactate (e.g. *Lactobacillus acidophilus*) maintain a tonic level of lactic acid in the rumen, which has the potential to stimulate microorganisms that utilize lactic acid (Nocek *et al.*, 2002).

The data confirmed that Y displayed the highest ($P < 0.05$) values for the differential species and total count at zero hour sampling time of feeding followed by C, while G had the lowest ($P < 0.05$) values. It seems that garlic powder diet decreased ($P < 0.05$) the count ($\times 10^5/mL$) of *Entodinium sp.*, *Epidenium sp.* and *Diplodinium sp.* as compared with control diet. Where, yeast supplement may have factors that encourage increasing the previous species to make record the highest ($P < 0.05$) values for the same order of protozoa species.

The present results of C group were higher than that found in goats fed diets with 70:30% concentrate to roughage ratio (Aziz *et al.*, 2018), this may be due to sheep was higher than goats in protozoa count as reported by Baraka (2012). With regard to protozoa number, it is clear the reduction effect on garlic powder in microbial activities. The antimicrobial properties of the aromatic plants are attributed in part to essential oils (Panghal *et al.*, 2011). Similar results were found by Nassar *et al.* (2017) who clarified that the addition of garlic powder or oil in rations of Barki sheep reduces ($P < 0.05$) the population of protozoal. Concerning yeast supplementation effect, the results were in accordance with those of Brossard *et al.* (2006) who stated that yeasts tended to increase the ruminal protozoal population ($P < 0.1$). Contrary

to current results, Hernández *et al.* (2009) showed that the addition of yeast to lambs fed early and mature orchard grass altered ruminal protozoa without affecting feed intake, total tract digestion and N balance.

Table (9): Total count and differential of rumen protozoa at zero time of feeding lambs and *in vitro* gas yield and kinetics for diets enriched with yeast and/or garlic.

Item	Experimental diet				SEM	P value
	C	Y	G	YG		
Protozoal Differential Count (x 10 ⁵ /mL)						
<i>Entodinium sp.</i>	6.70 ^b	8.17 ^a	4.44 ^d	5.10 ^c	0.43	0.048
<i>Epidenium sp.</i>	3.71 ^b	6.13 ^a	2.68 ^d	2.99 ^c	0.36	0.044
<i>Diplodinium sp.</i>	0.66 ^b	1.97 ^a	0.51 ^c	0.54 ^c	0.17	0.049
<i>Polyplastron sp.</i>	0.48	0.47	0.45	0.44	0.18	0.611
Total (x 10 ⁵ /mL)	11.55 ^b	16.74 ^a	8.08 ^d	9.07 ^c	0.44	0.050
Total gas yield; TGY (ml/200mg DM)						
TGY _{3h}	16.13 ^a	15.78 ^a	12.27 ^b	15.28 ^a	0.38	>0.001
TGY _{6h}	24.53 ^a	23.03 ^a	17.53 ^c	18.03 ^c	0.21	>0.001
TGY _{12h}	28.03 ^a	27.03 ^b	21.53 ^c	21.53 ^c	0.20	>0.001
TGY _{24h}	30.53 ^a	28.28 ^b	19.53 ^d	21.53 ^c	0.53	>0.001
TGY _{48h}	32.2 ^a	30.87 ^b	27.08 ^c	24.53 ^d	0.40	>0.001
PF*	1.93 ^c	2.81 ^b	3.75 ^a	3.08 ^b	0.10	<0.001
Kinetic constants:						
A	2.78 ^b	3.78 ^b	11.87 ^a	11.96 ^a	0.72	<0.001
B	28.41 ^a	25.79 ^a	18.63 ^b	11.95 ^c	0.83	<0.001
a+b	31.19 ^a	29.57 ^a	30.50 ^a	23.91 ^b	1.13	0.007
C	0.219 ^a	0.215 ^a	0.041 ^c	0.117 ^b	0.02	<0.001
Methane yield:						
CH ₄ yield _{24h} , ml	18.57	18.41	8.20	11.74	0.22	<0.001
CH ₄ : TGY ratio	60.84	65.09	41.97	54.55	0.51	>0.001
CH ₄ energy (MJ/d)	5.30	5.30	2.39	3.34	-	-
CH ₄ energy/GEI %	21.15	20.94	9.30	13.33	-	-
Microbial protein synthesis:						
MCP (g/kg TDOM)	71.14 ^c	95.66 ^a	88.04 ^b	80.02 ^b	0.96	<0.001

*The ratio of true digestible organic matter (mg) to gas volume (milliliters in 24 h); a, b and c are gas yield constants.

SEM: standard error of the mean, P value: probability value

a, b and c means at the same row with different superscript letters are significantly ($P < 0.05$) different.

Fermentation gas yield and kinetic constants:

Gas production reflects all nutrients fermented (soluble as well as insoluble) and fractions that are not fermentable do not contribute to the gas yield (GY). The amount of gas produced is influenced by the rate of fermentation of carbohydrate, the molar proportions of the VFA and the amount of VFA produced (Dijkstra *et al.*, 2005). Differences in the 'a' and 'c' parameters indicate different fermentation patterns, suggesting that Y is fermented more rapidly and to a greater extent. The cumulated gas production (ml/200 mg DM) for each diet the kinetics values of gas production models are given in Table (9). The total gas yield (TGY, ml/200 mg DM) increased ($P < 0.001$) with developing fermentation time up to 48h for all experimental diets except for G that dropped GY at 24h. The diet of Yeast (Y) increased ($P < 0.05$) the b fraction, as also shown by the C-diet, whereas it was lower ($P < 0.001$) with the G alone and the combination YG. In contrast, the intercept fraction (a) increased ($P < 0.001$) in G and YG diets. The later diets showed lower rate of fermentation (c) as compared with C and Y diets. The total gas yield at 24h of incubation of Y, G and YG found to be lower than those found for C diet. The present results are lower than those reported for barley (64-71 ml), wheat (60-73 ml) and corn grains (60-82 ml) by Getachew *et al.* (2002), these finding might be due to applying the gas production technique herein with total mixed rations. The high gas yield as well as the OMD value of the respective additive groups could be attributed to a higher fermentation process resulting by intact the feed additives with the basal diet. Potential gas production (a) was not significantly different in study of Ikyume *et al.* (2018) and ranged between 15.54-27.65 ml. But, the highest value 27.65 ml was observed in the control group compared to all supplemented groups that having a similar value of 15.54 ml. The constant gas production rate (c) was also observed to be non-significant ($P > 0.05$) across treatment groups and ranged from 1.22 and 1.73

ml/hr. The highest gas production rate was observed in the 1% garlic powder group (1.73 ml/h) with the least value recorded in the control group (1.22 ml/h). Incubation time (t) was not significant ($P>0.05$) across the treated groups and ranged from -5.96 to -0.7h. The highest incubation time was recorded in the garlic powder 0.5% group with the least time found to be in the control group (Ikyume *et al.*, 2018).

Higher PF values (Table 9) in respective additive diets especially G reflected high ratio of OM fermented to gas production as compared with C diet. According to Geneviève *et al.* (2018), the quantity of gas produced in the rumen is inversely correlated with microbial yield, which means that the PF value reflects changes in microbial biomass yield. Favorable rumen digestion responses to yeast culture feeding in ruminants include increased degradation of DM and OM (Kamel *et al.*, 2004) and stimulation of total and cellulolytic bacterial numbers (Newbold *et al.*, 1995). Based on mean growth, there was a tendency for *S. cerevisiae* supplementation to increase rumen bacteria, whereas control treatment decreased rumen bacteria (Riyanti and Evvyernie, 2016).

Methane yield:

Data in Table (9) obviously showed that diets contained garlic powder (G and YG) were most effective in reduction of energy lost when expressed as a ratio of methane production to gross energy intake. Ikyume *et al.* (2018) found that garlic inclusion had no ($p>0.05$) influence on all the *in vitro* digestibility kinetics measured except methane (CH_4) output, Gas volume (GV), CH_4 : GV ratio, metabolizable energy, and short chain fatty acids. They also found that methane to gas volume ratio expressed in percentage significantly ($P<0.05$) decreased with increasing amount of garlic powder supplementation (0, 0.5, 1.0 and 1.5%). While the highest value of 75 % was observed in the control group, the garlic powder 1.5% group had the least value of 19.02 %. *In vitro* dry matter digestibility (IDMD) was not statistically ($P> 0.05$) significant, GP 0.5% group had numerically higher value (68.11%) while the control had the least value of 65.23%. The addition of garlic powder to the diet of rams considerably reduced production of CH_4 by 38% (Kim *et al.*, 2018).

Microbial protein synthesis:

Microbial protein (MCP) synthesized in the rumen provides the majority of the protein supplied to the small intestine of ruminants, which represents 50 to 80% of total absorbable protein (Firkins *et al.*, 2007). The microbial protein was affected by feed additives (Table 9). Where, MCP synthesis was more enhanced ($P<0.001$) by yeast than other treatments. However, Wanapat *et al.* (2008 and 2013) showed opposite results to this finding that garlic supplementation did not produce significant changes in the synthesis of microbial proteins and/or urinary purine derivatives. The multiplication of number of bacterial by yeast supplementation may increase the rate of fermentation of the substrate and the synthesis of MCP (Ryan and Gray, 1989). The addition of yeast (Y) stimulated rumen microbial growth through the use of specific soluble growth factors such as organic acids, B vitamins and provided amino acids (Waldrup and Martin, 1993). The positive effect of the addition of SC in the present study is consistent with the findings of many workers (Guedes *et al.*, 2008). Relating garlic addition, Ikyume *et al.* (2018) stated that the number of bacteria increased ($P> 0.05$) numerically and consequently increased the microbial protein as the level of garlic powder supplementation increased. The highest count was recorded in the garlic powder 1.5% group while the least value was observed in the control group.

CONCLUSION

It could be concluded that using feed additive such as dry yeast (6 gm/h/d) or garlic powder (40 gm/h/d) or its combination (3gm plus 20 gm) in finishing diets of lamb tended to increase digestibility for most of nutrients, increasing nutritive value as TDN and appeared to increase the daily gain. Furthermore these feed additives have enhanced feed efficiency and improved the immune status of animals. Although the addition of garlic powder alone is restricted by a low price case, it is most effective in reducing energy loss when expressed as a ratio between methane production and gross energy intake and also proved effective in reducing methane emissions from sheep and therefore contributing to global warming.

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تأثير الخميرة والثوم أو مخلوطهما بإضافات غذائية على الأداء وتخميرات الكرش والحالة المناعية للحملان المغذاة على علائق ناهية

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تهدف هذه الدراسة الى تقييم تأثير الخميرة ومسحوق الثوم الجاف ومخلوطهما على الأداء وتخميرات الكرش والحالة المناعية للحملان المغذاة على عليقة ناهية، لذا استخدمت أربعة مجموعات من الحملان (سبعة حملان/ مجموعة) عمر 8 شهور وبمتوسط وزن 35.8 كجم. غذيت الحملان على علف مركز بنسبة 70% من احتياجات النمو الكلية مع تقديم دريس برسيم حتى الشبع. وكانت المعاملات الغذائية كالتالي: عليقة أساسية (علف مركز + دريس) بدون إضافة (عليقة المقارنة) ، عليقة أساسية مع إضافة 6 جرام خميرة/ رأس/ يوم ، عليقة أساسية مع إضافة 40 جرام ثوم جاف/ رأس/ يوم وعليقة أساسية مع إضافة مخلوط من 3 جرام خميرة + 20 جرام ثوم جاف/ رأس/ يوم

وأوضحت النتائج ما يلي:

- أدت الإضافات الغذائية الى تحسين (مستوى معنوية 5%) معاملات هضم المادة الجافة، المادة العضوية، البروتين الخام، الألياف الخام، والمستخلص الخالي من النيتروجين. وأظهرت مجموعة الثوم أعلى القيم بينما كانت أقل القيم لمجموعة المقارنة.
- أظهرت عليقة الثوم الجاف أعلى (مستوى 5%) قيمة غذائية في صورة مركبات كلية مهضومة (73.56%) بينما كانت عليقة المقارنة هي الأقل (69.20%). أما القيمة الغذائية للعلائق في صورة بروتين مهضوم فلم تتأثر معنويا (مستوى معنوية 5%) بالإضافات الغذائية موضع الدراسة وتروحت القيم بين 11.81 و 12.27%.
- تفوقت مجموعات الإضافة معنويا على مجموعة المقارنة في معدل النمو وكانت القيم 180، 184، 186 ، 160 جم/يوم لمجموعة الخميرة، الثوم، مخلوط الخميرة مع الثوم، المقارنة على التوالي.
- تشابهت الإضافات في تحسين الكفاءة التحويلية مقارنة بمجموعة المقارنة.
- حققت مجموعة مخلوط الإضافتين نيتروجين محتجز بمقدار 15.2% أعلى من مجموعة المقارنة بينما تشابهت مجموعتي الخميرة والثوم وكانت القيم بين مجموعتي المقارنة ومخلوط الإضافة.
- أدت الإضافات موضع الدراسة الى تحسين (مستوى معنوية 5%) تركيز الجلوبيولينات المناعية في الدم مقارنة بمجموعة المقارنة. نستنتج من هذه الدراسة ما يلي: ان استخدام 6 جرام خميرة/ رأس/ يوم أو 40 جرام ثوم جاف/ رأس/ يوم أو مخلوط منهما بمعدل 3 جرام + 20 جرام ثوم/ رأس/ يوم كإضافات غذائية في العليقة الناهية للحملان النامية أدت الى تحسين هضم العناصر الغذائية والقيمة الغذائية في صورة مركبات كلية مهضومة بالإضافة الى تحسين معدل النمو اليومي ورفع الحالة المناعية للحملان، ولكن استخدام مسحوق الثوم بمفرده يكون فقط عند رخص ثمنه. والجدير بالملاحظة هوالتاثير الإيجابي للثوم في خفض إنتاج الميثان وبالتالي خفض الفقد في طاقة الغذاء مما يؤدي الى تقليل الإحتباس الحراري في البيئة.