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Effect of Herbal Mixture with or without Probiotic on Digestion, Blood Metabolites, Growth Performance and Carcass Characteristics of Barki Lambs

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ABSTRACT

This study aimed to investigate the effect of herbal mixture with or without probiotic yeast on digestion, blood metabolites, immunity and antioxidant status, growth performance and carcass characteristics. Fifteen early weaned male Barki lambs (13.5 ± 0.82kg) were divided randomly into three groups (n=5/group) as follows: Group one (G1) was fed on basal diet that was consisted of concentrate feed mixture (CFM) and berseem hay as control; group two (G2) was fed on basal diet with herbal mixture (garlic, ginger, cumin and turmeric) at 1% of dry matter intake (DMI) and group three (G3) was the fed like G2 plus five gram per head of probiotic yeast (*Saccharomyces cerevisiae*). Animals were fed for seven months and twenty days. The combination of herbal mixture and yeast (G3) increased nutrients digestibility, nutritive value, growth rate, serum thyroid hormone, immunoglobulins and total antioxidant ($P< 0.05$). Meanwhile, the same group (G3) decreased predicted methane intensity, and carcass chilling loss ($P< 0.05$). Herbal mixture (G2) increased brightness and yellowness of meat color ($P< 0.05$). It could be concluded that the combination of herbal mixture with yeast made a positive synergistic effect on the performance of Barki lambs.

Keywords: Herbal mixture, probiotics, yeast (*Saccharomyces cerevisiae*), immunoglobulins, methane intensity, carcass characteristics and Barki sheep.

1. Introduction

Animal products (meat and milk) are vital sources of highly nutritional nutrients (protein, energy, minerals and vitamins) for human health and growth. Increasing global demand for animal products in response to the growing human population makes the required sustainability approach of livestock production in a concerned challenge. This is because of increasing concern of antimicrobial resistance (AMR) and methane production which increase with expanding ruminant production worldwide (Pulina *et al*., 2017; Van Eenennaam, 2024; Matheou *et al*., 2025).

Misuse of antibiotics globally, especially as growth promoters in livestock production, is rising the threat of AMR to medical antibiotic treatments which puts health care both for humans and animals in severe danger. So, using alternatives to antibiotics such as herbal mixture, probiotics, prebiotics, enzymes,...etc., is highly recommended (Seal *et al*., 2013 ; Aslam *et al*., 2021 and Matheou *et al*., 2025).

Ruminants produce methane during the fermentation of feed in the rumen. Methane wastes about 2-12% of gross energy ingested by ruminants. Moreover, methane is one of the potent greenhouse gases (its warming potential equaled 28 times that of CO_2) which are responsible for climate change phenomena and its negative effects such as global warming (Johnson and Johnson, 1995; Davison *et al*., 2020; Belanche *et al*., 2023).

Herbal mixture consists of many herbal plants such as garlic, ginger, cumin, and turmeric which have phytochemical compounds (tannins, flavonoids, saponins, and alkaloids) with beneficial effects on animal performance, when used in an appropriate way, such as an immune stimulant, anti-inflammatory, antioxidant, antimicrobial, antiparasitic, methane inhibitor, fertility improvement and

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growth promoter (Arafa *et al*., 2023; Abd El-Hamid *et al*., 2023; Ghandour *et al*., 2025; Rabee *et al*., 2025).

Probiotic is a live microorganism like yeast (*Saccharomyces cerevisiae*) and bacteria (*Lactobacillus acidophilus* and *Bacillus subtlis)* that are used to induce preferred changes in animal digestion and performance. Yeast enhances animal performance through stabilizing the rumen environment, elevating nutrients digestibility, and mitigating heat stress (Zhang *et al*., 2022; Kholif *et al*., 2024).

Studies that discuss both the combined effects of herbal mixture and probiotics are very rare. So, the objective of this study was to investigate the effect of a herbal mixture with or without probiotic (yeast) on feed intake, digestion, nutritive value, growth rate, blood metabolites, immunity, antioxidant and carcass characteristics.

2. Materials and Methods

2.1. Ethical approval

Animal management, handling, and slaughtering were approved by the Animal Care and Use Committee of the Animal and Poultry Production Division, Desert Research Center (DRC), Egypt.

2.2. Animals and diets

The current study was carried out at Maryout Research Station, Desert Research Center (DRC), southwest of Alexandria, Egypt. Fifteen early weaned male Barki lambs (13.5 ± 0.82kg) were randomly divided into three groups (n=5/group) and fed for 230 days (7 months and 20 days). Group one (G1) was fed on basal diet as concentrate feed mixture (CFM) and berseem hay (*Trifolium alexandrinum*) as control, group two (G2) was fed on the basal diet like G1 with herbal mixture at 1% of dry matter intake (DMI) and group three (G3) was fed like G2 but with five gram probiotic yeast (*Saccharomyces cerevisiae*) per animal according (Kewan *et al*., 2021).

Table 1: Chemical analysis of feed ingredients and the whole ration (on DM basis, %).

Item (%)	Concentrate feed mixture*	Hay	Ration**
DM%	90.44	86.62	89.01
OM%	92.98	87.12	90.78
Ash%	7.02	12.88	9.218
CP%	17.01	13.02	15.52
EE	2.08	1.69	1.933
CF	5.58	28.93	14.34
NFE	68.30	43.48	58.99
NDF	37.63	51.16	42.70
ADF	10.73	37.19	20.66
CHO**	73.89	72.41	73.33
NFC**	36.26	21.25	30.63

*Concentrate feed mixture (CFM) was consisted of yellow corn (50%), soybean meal (20%), wheat bran (22%), undecorticated cotton seed meal (5.0%), limestone (1.2%), common salt (0.9%), sodium bicarbonate (0.3%), premix (0.2%), anti-toxins (0.2%), yeast extract (0.2%). **Calculated values. DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; CF: crude fiber; NFE: nitrogen free extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; CHO: total carbohydrate; NFC: non fiber carbohydrate. CHO% = 100 – (CP% + EE% + Ash%) according to Fox *et al*. (2004). NFC% = 100% - (CP% + NDF% + EE% + Ash%) according to Hall (2003).

Chemical analysis of feed ingredients and the whole ration are shown in Table 1. The herbal mixture consisted of commercial air-dried powder of garlic (*Allium sativum*), cumin (*Cuminum cyminum*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) with equal portions (1:1:1:1) according to Rabee *et al*. (2024). Probiotic yeast (*Saccharomyces cerevisiae*) was an active dry yeast (2 x 10^7 colony forming units (CFU/g)) is a commercial product of the ANGEL yeast company, Egypt. Yeast and herbal

mixture were not mixed until they were mixed well with CFM prior to feeding daily to avoid any unfavorable conditions for the live probiotic yeast.

Animals were fed on CFM and berseem hay at 2.5% and 1.5% of animal live body weight (LBW), respectively in group feeding according to NRC (2007). CFM was offered two times per day (at 9:00 AM and 3:00 PM) to avoid a rapid fall in rumen pH according to Kewan *et al.* (2021); Hamdon *et al.* 2024). Water was available twice a day. Feed offered and refusals were weighed and recorded daily. Animals were weighed every two weeks and feeds and herbal mixture were adjusted according to the changes in the LBW.

2.3. Nutrients digestibility

Before the end of the experiment, four animals from each group were used to determine nutrient digestibility using stainless steel metabolic crates for seven days as a preliminary period followed by seven days as a collection period according to Schneider and Flatt (1975). Feeds, water and refused feeds were recorded daily and composite samples were kept for further lab analysis. Feces were collected and weighed daily, and 10% was dried to constant weight then ground and kept for further lab analysis. Gross energy, total digestible nutrients (TDN), digested energy (DE), metabolizable energy (ME), methane production were calculated by different equations. Predicted methane production (g/d) was converted to energy (MJ) according to Ku-vera *et al*. (2013).

2.4. Rumen sampling and fermentation parameters

Rumen liquor was withdrawn using a stomach tube at 3 hours after feeding. A portable calibrated digital pH meter was used to measure pH immediately. The rumen liquor samples were filtered through three layers of cheese cloth and put in plastic bottles and preserved by using drops of toluene and paraffin oil and kept at -20°C for determination of total volatile fatty acids (TVFA's) and ammonia–nitrogen (NH_3-N) which were determined using the steam distillation method and micro-Kjeldahl's method as described by Annison (1954) and AOAC (1990), respectively.

2.5. Chemical analysis

Proximate analysis in term of dry matter (DM), crude protein (CP), crude fiber (CF), crude fat as extracted by ether extract (EE) and ash of feeds, orts and feces samples were analyzed according to AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest and Robertson (1985) using ANKOM Technology (ANKOM Technology, New York, United States). The antioxidant activity of the herbal mixture as 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Burits and Bucar (2000). Flavonoid fractions of the herbal mixture were determined using high-performance liquid chromatography (HPLC) according to the method of Biswas *et al*. (2013).

2.6. Blood serum parameters

Blood samples were collected before the end of the experiment from the jugular vein before morning feeding and allowed to stand at room temperature for about 2-3 hours then centrifuged at 3000 rpm for 20 minutes to get serum samples then stored at -20°C for further analysis. Serum total protein, albumin, triglycerides, total cholesterol, creatinine, urea, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and total antioxidant capacity (TAC) were determined by using colorimetric methods according to the kits manufacturer recommendations (Spectrum Diagnostics and Bio-diagnostic, Egypt). Serum thyroid hormones (thyroxine (T4) and triiodothyronine (T3) concentrations were determined by a competitive immunoassay kit using iFlash immunoassay analyzer (Shenzhen Yhilo Biotech. Co. Ltd., China). Serum immunoglobulin (M and G) concentrations were determined by turbid-metric immunoassay using Quantia IgM and IgG kit (Verna Industreal Estate, Vrna, Goa-403722, India).

2.7. Carcass characteristics and meat color

At the end of the experiment, all lambs were slaughtered according to the Halal Islamic method (by cutting the neck veins and trachea) after fasting for 12 hours and the weight of animals was recorded as fasting weight. After slaughter and complete bleeding, the weight of the head, feet, pelt, testes, liver, heart, kidneys, spleen, full and empty digestive tract, and total internal fats (abdominal and kidney fat)

were recorded. Hot carcass weight was recorded, and dressing percentage was calculated. The carcasses were chilled for 12 hours under 4°C then weighed and chilled loss was calculated. The chilled carcass was divided into neck, shoulders, racks, loin, flank, legs, and tail. The best ribs (9, 10 and 11th) cuts were separated into their physical components (lean meat, fat and bone) and weighed according to Ghandour (2015) and Zayed *et al*., (2022). The area of the eye muscle (*Longissimus dorsi*) was determined by tracing the muscle on semitransparent waxed paper (calc) and the area was measured by polar plane meter (Planix5- Tawya Tech. Japan). Meat color in terms of lightness (L*), redness (a*) and yellowness (b*) was determined by Chroma meter (Konica Minolta model Cr 410, Japan) according to Luciano *et al*. (2009).

2.8. Histology

Autopsy samples were taken from eye muscle tissues in different groups of slaughtered Barki lambs to conduct the histological examination. Samples were fixed in a 10% formol saline, then washed, dehydrated in ethyl alcohol of different grades, cleared in methyl benzoate, and embedded in paraffin wax at 56°C in a hot air oven for 24 hours. Blocks were processed using standard procedures. Sections (5-µm thick) were stained with hematoxylin and eosin (H&E) and examined microscopically according to Bancroft and Gamble (2013).

2.9. Calculation

Total carbohydrate (CHO):
CHO% = 100 – (CP% + EE% + Ash%) according to Fox *et al*. (2004).

Non-fiber Carbohydrates (NFC):
NFC% = 100% - (CP% + NDF% + EE% + Ash%) according to Hall (2003).

Gross energy equation:
GE (MJ/KgDM) = 0.0176 OM (g/kg) + 0.0064 CP(g/kg) + 0.0214 EE(g/kg) according to SCA (1990).

Methane prediction equation:
CH4 (g/d) = 4.72+ 0.116*BW + 11.8*DMI– 0.0440*OMD

Where BW was the animal's live body weight (Kg), DMI: dry matter intake (Kg), OMD: organic matter digestibility percentage according to Belanche *et al*. (2023).

Digested energy was calculated from total digestible nutrients (TDN)
TDN% = DE/ 0.04409 according to NRC (2007).

DE: digestible energy (Mcal/KgDM)
Metabolizable energy = 0.82 x DE, according to NRC (2007).
Carcass weight loss index %= (Hot carcass weight (g) – Cold carcass weight (g))/Hot carcass weight × 100. According to Vaz *et al.* (2025).

2.10. Statistical analysis

Differences among groups were checked using SAS (1996) throughout the general linear model (GLM) procedure (one way analysis of variance) at $P<0.05$. Duncan's new multiple range test (Duncan, 1955) was used to compare among means. The following statistical model was adopted:

$$Yij = \mu + Gi + eij$$

Where: Yij = observation, µ = over all mean, Gi = the effect of the groups (G = 1, 2, 3; 1=control group; 2= herbal mixture group; 3= herbal mixture with probiotic group), and eij = experimental error, assumed to be randomly distributed (0, σ2).

3. Results and Discussion

3.1. Phyto chemical constituents of herbal mixture

The active flavonoid compounds of herbal mixture (garlic, cumin, ginger and turmeric) were summarized as follows: Rutin (0.10 mg/g), Hisperidin (5.72 mg/g), Apeginin (8.92 mg/g), Kampferol (21.17 mg/g) and Quercetin (70.53 mg/g). The antioxidant activity as (DPPH) scavenging activity reached 41.38%.

3.2. Feed intake, digestibility and nutritive value.

The effect of herbal mixture with or without probiotic yeast (*Saccharomyces cerevisiae*) on feed and water intake, digestibility and nutritive value during the digestibility trial are presented in Table 2. Dry matter intake (DMI) and water intake were non-significant (P >0.05) and similar among the three groups. These results agreed with Kewan *et al*. (2021) who reported that feed and water intake were non-significant among control, phytogenic additive (garlic) and with probiotic(yeast) groups. Moreover, Burt *et al*. (2023) reported that adding yeast to the ration of sheep did not alter feed intake (1.12 and 1.19 kg/d) versus control (without yeast). The same observation was reported by El-Naggar and Ibrahim (2018) and Abo Bakr (2025).

It could be summarized from Table 2 the combination of yeast with a herbal mixture in the third group (G3) resulted in the highest nutrients digestibility and subsequently nutritive value among the experimental groups.

The values of dry matter digestibility (DMD) and organic matter digestibility (OMD) were significant (P< 0.05), and the highest values were found in the herbal mixture with yeast group (G3) by 5.3 and 5.2% on average over G1 and G2 groups, respectively. Similar results were obtained by Kewan *et al*. (2021).

Table 2: Effect of herbal mixture (HM) and HM with yeast on feed intake, water intake, nutrients digestibility and nutritive value of Barki lambs during digestibility trial.

Item	G1	G2	G3	±SE	P value
DMI (kg)	1.504	1.468	1.456	0.043	0.915
OMI (kg)	1.366	1.334	1.326	0.040	0.923
Water intake (l/d)	4.889	5.053	4.232	0.239	0.368
Water/ DMI	3.275	3.449	2.910	0.147	0.339
Digestibility (%)					
DMD	71.98^{b}	72.69^{b}	76.15^{a}	0.736	0.026
OMD	72.62^{b}	72.86^{b}	76.53^{a}	0.698	0.017
CDP	72.34^{b}	71.31^{b}	76.60^{a}	0.876	0.013
EED	80.15^{b}	78.10^{b}	85.80^{a}	1.344	0.033
CFD	34.83^{b}	36.84^{b}	46.19^{a}	1.792	0.005
NFED	81.49	81.47	83.52	0.447	0.086
NDFD	57.45^{b}	57.69^{b}	64.09^{a}	1.174	0.013
ADFD	44.34^{b}	44.26^{b}	53.01^{a}	1.496	0.006
Nutritive value (%)					
TDN	68.42^{b}	68.55^{b}	72.61^{a}	0.7194	0.007
DCP	11.25^{b}	11.11^{b}	12.03^{a}	0.147	0.005

Means followed by different superscripts (a and b) within the same row are significantly different (P≤0.05).
G1: Control, G2: Herbal mixture and G3: Herbal mixture with yeast. SE (standard error). DMI: dry matter intake. OMI: organic matter intake. DMD: dry matter digestibility. CPD: crude protein digestibility. EED: ether extract (crude fat) digestibility. CFD: crude fiber digestibility. NFED: nitrogen free extract digestibility. NDF: neutral detergent fiber digestibility. ADF: acid detergent fiber digestibility. TDN: total digestible nutrients. DCP: digestible crude protein.

This enhancement in DMD and OMD% and subsequently other nutrients digestibility may be due to the role of yeast (*Saccharomyces cerevisiae*) on the rumen. Yeast maintained anaerobiosis of the rumen environment (to be strictly anaerobic) by consuming any oxygen particles found in the rumen

that came from recently ingested feeds and dissolved oxygen in drinking water. Also, yeast maintained low redox potential of the rumen environment that increased with a low roughage and low rumen pH. That stimulates growth, numbers and the fermentation rate of rumen microorganisms (Fonty and Chaucheyras-Durand, 2006; Firkins and Michell, 2023).

In the same pattern, crude protein digestibility (CPD%) and ether extract (crude fat) digestibility (EED%) were significant ($P< 0.05$) and the highest value was found in group (G3) by 6.65% and 8.45% on average over the other groups, respectively. This increase was due to the improvement in DMD and OMD%. Moreover, yeast (*Saccharomyces cerevisiae*) produces protease and lipase enzymes during fermentation (Shirazi *et al*., 1998; Maturano *et al*., 2015). Also, ruminal protease enzyme activity increased with probiotic additives compared to the control group (Sheikh *et al*., 2022). That may be enhanced CPD% and EED%.

Fiber digestion (CFD, NDFD and ADFD%) values were significant ($P< 0.05$) and the highest values were found in herbal mixture with yeast group (G3).

These results agreed with Kewan *et al*. (2021) who reported that the Barki sheep supplemented with garlic and yeast group was higher in fiber digestibility than the control. The same observation of the positive effect of probiotic yeast on fiber digestion was reported by Farghaly and Hamdon (2018).

Generally, enhanced fiber digestibility (CF, NDF and ADF) may be because yeast supports anaerobiosis and low redox potential in the rumen environment that stimulate colonization and degradation of rumen fibrolytic microorganisms (Fonty and Chaucheyras-Durand, 2006; Firkins and Michell, 2023).

Also, yeast enhanced fiber degradation despite high concentrate diet through stabilizing rumen pH by competing with lactate producing bacteria (*Streptococcus bovis*) on the carbon source (glucose) and stimulating lactate utilizing rumen bacteria (*Selenomonas ruminantium*) to consume more lactate by releasing malic acid and thiamin as growth factors (Nisbet and Martin, 1991; Fonty and Chaucheyras-Durand, 2006).

High nutrients digestibility in G3 group was reflected in the higher significant ($P< 0.05$) value of TDN (72.34%) than G1 and G2 groups. The same pattern was observed in DCP which was significant ($P< 0.05$) and higher in the G3 group (12.03%) than in the other groups.

3.3. Energy utilization and predicted methane production

Energy utilization and methane production data are shown in Table 3. Gross energy intake (GEI) was non-significant and almost the same among groups and averaged value was 17.42 MJ/kgDM, and this was due to the feed intake being non-significant and close to each other among the experimental groups (Table 2). The values of DE and ME were significantly ($P< 0.05$) higher in G3 by 5.52 and 5.60% on average than the G1 and G2 groups. Metabolizability values were significant ($P< 0.05$) and higher in the G3 than other groups. DE and ME were positively affected by OMD and TDN% (Table 2). Predicted methane production values were non-significant and numerically tended to be the lower in G2 and G3 groups by 2.50 and 2.10% from the control group (G1), respectively. Herbal mixture with yeast group (G3) saved more energy as lower methane than the average of the other groups (G1 and G2) by 7.30, 9.20 and 11.22% from GE, DE and ME, respectively.

The presence of flavonoid compounds in herbal mixture such as quercetin in the present study may decrease ruminal methane production (Ghandour *et al*., 2025; Ku-Vera *et al*., 2020). Additionally, herbal mixture and yeast increased ruminal propionic acid and decreased ruminal protozoal count in parallel with low methane production (Kewan *et al*., 2021; Rabee *et al*., 2025). Ruminal microorganisms that produce propionic acid compete with methanogenic archaea on metabolic hydrogen utilization (Matheou *et al*., 2025). Moreover, methanogenic archaea are hosted (live on and within) by rumen protozoa to protect them from oxygen and metabolic hydrogen uptake. Therefore, methanogenesis decreased as protozoal count decreased (Newbold *et al*., 1995; Dai *et al*., 2022).

Intensity of predicted methane production per kg live body weight (CH4 g/KG LBW) was significant ($P< 0.05$) and lower in G3 by 16.52 and 13.75% than in the G1 and G2 groups, respectively. This low methane intensity in the G3 is related to low predicted methane production and higher growth rate and high feed efficiency over the other groups (Table 5) and this is supported by the negative correlation between feed efficiency and *Methanobrevibacter*, which is considered the main methane producer in the rumen (Bharanidharan *et al*., 2018; Rabee *et al*., 2025).

Additionally, increasing consumers' awareness against negative effects of methane emission on animal productivity and climate change may increase the choice of meat produced from low methane cattle (Davidson *et al*., 2025). That may encourage farmers to produce meat with low methane intensity.

Table 3: Effect of herbal mixture (HM) and HM with yeast on feed energy utilization and methane production of Barki lambs.

Item	G1	G2	G3	±SE	P value
GE (MJ/kgDM)	17.40	17.41	17.45	0.01	0.22
DE (MJ/kgDM)	12.60[b]	12.63[b]	13.36[a]	0.13	0.010
ME (MJ/kgDM)	10.33[b]	10.35[b]	10.97[a]	0.11	0.010
ME/GE	0.59[b]	0.60[b]	0.63[a]	0.01	0.004
Methane (g/d)	23.67	23.18	23.09	0.68	0.940
CH_4/GE (%)	7.51	7.35	7.30	0.21	0.930
CH_4/DE (%)	10.38	10.17	9.20	0.35	0.370
CH_4/ME (%)	12.66	12.4	11.22	0.42	0.370
Methane intensity (CH4, g/kg LBW)	229.42[a]	223.07[a]	191.53[b]	5.80	0.0120

Means followed by different superscripts (a and b) within the same row are significantly different (P≤0.05).
G1: Control, G2: Herbal mixture and G3: Herbal mixture with yeast. SE (standard error). GE (MJ/KgDM) = 0.0176 OM (g/kg) + 0.0064 CP(g/kg) + 0.0214 EE(g/kg) according to SCA (1990) *Methane (CH4 (g/d) = 4.72+ 11.8*DMI+ 0.116*BW- 0.0440*OMD) according to (Belanche *et al*., 2023) then converted into energy (MJ) according to Ku-vera *et al*. (2013). GE: gross energy; DE: digested energy; ME: metabolizable energy; CH4: methane; LBW: live body weight.

3.4. Rumen fermentation parameters

Rumen fermentation parameters are shown in Table 4. Rumen pH values were significant (P< 0.05), and numerically lower in the G3 and G2 groups by 0.30 and 0.20 pH points than the control (G1). These differences in pH may be due to high digestion of organic matter (Table 2) and higher values of total volatile fatty acids (TVFA's) that were recorded in the G3.

Table 4: Effect of herbal mixture (HM) and HM with yeast on rumen fermentation parameters of Barki lambs.

Item	G1	G2	G3	±SE	P value
pH	6.68[a]	6.48[b]	6.40[b]	0.05	0.020
TVFA's (m. equiv./100 ml)	7.97	7.94	9.56	0.40	0.150
NH_3-N (mg/100 ml)	19.12	21.36	23.50	1.03	0.230

Means followed by different superscripts (a and b) within the same row are significantly different (P≤0.05).
G1: Control, G2: Herbal mixture and G3: Herbal mixture with yeast. SE (standard error). TVFA's: total volatile fatty acids; NH_3-N: ammonia nitrogen.

TVFA's concentrations were non-significant (P> 0.05) and numerically recorded a higher value in the G3 goup (9.56 m.equiv./100 ml) than the other groups. Increasing TVFA's concentration may be due to high digestion of organic matter and fiber (Table 2). Higher ruminal TVFA's concentrations were observed in yeast group than the control (Kewan *et al*., 2021; Farghaly and Hamdon, 2018). The role of yeast in the rumen is to support anaerobiosis and low redox potential that stimulates the growth and fermentation rate of rumen microorganisms and subsequently increased TVFA's concentrations (Fonty and Chaucheyras-Durand, 2006; Firkins and Michell, 2023). Also, the same pattern was observed in ammonia nitrogen (NH_3-N) concentrations, which were non-significant (P> 0.05) and numerically higher value was observed in the G3 group (23.50 mg/100 ml). This may be due to the higher crude protein digestibility (CPD%) than the other groups (Table 2). Additionally, ruminal protease enzyme activity was higher in the probiotic group than in the control group (Sheikh *et al*., (2022). The same pattern was observed in previous studies (Jurkovich *et al*., 2014; Liu *et al*., 2019 and Rabee *et al*., 2022).

3.5. Blood metabolites and immunity

Serum blood metabolites are shown in Table 5. The values of serum blood metabolites of the experimental groups (G1, G2 and G3) were in the normal range of healthy sheep according to Jackson and Cockcroft (2002). Serum total protein (p= 0.06) and albumin (P< 0.05) were higher in the (G3) group than the other groups and that may be due to the higher CPD% was in (G3) (Table 2). Improvement in protein digestibility reflected in improvement in blood protein (Asrar *et al*., 2023).

The concentrations of thyroxine (T4) were significantly (P< 0.05) higher in the G2 and G3 groups than the control group, and the concentrations of triiodothyronine (T3) had the same line but without significance (P> 0.05). These findings agreed with a high level of thyroid hormones in ruminants that were supplemented with yeast (Yousef *et al*., 1996; Zhang *et al*., 2022).

Table 5: Effect of herbal mixture (HM) and HM with yeast on serum blood metabolites and immunity of Barki lambs.

Item	G1	G2	G3	±SE	P value
Total protein (g/dl)	6.18	6.24	6.70	0.10	0.06
Albumin (g/dl)	2.20^{b}	2.18^{b}	2.40^{a}	0.04	0.02
Globulin (g/dl)	3.98	4.06	4.30	0.08	0.25
A/G ratio	0.56	0.54	0.56	0.01	0.71
Urea (mg/dl)	35.15	41.74	40.85	1.71	0.25
Creatinine (mg/dl)	0.89	0.89	0.91	0.02	0.90
GPT (U/L)	21.40	26.00	21.00	1.44	0.31
GOT(U/L)	105.60	120.40	112.25	3.97	0.34
Cholesterol (mg/dl)	41.62	35.66	44.05	3.05	0.55
Triglycerides (mg/dl)	19.66	19.72	20.28	1.07	0.97
Triiodothyronine (ng/dl)	86.60	93.10	93.70	2.179	0.35
Thyroxine (µg/dl)	5.38^{b}	7.44^{a}	6.97^{a}	0.277	0.002
IgM(mg/dl)	51.90^{b}	66.33^{a}	77.30^{a}	3.085	0.001
IgG(mg/dl)	340.17	342.25	344.40	5.913	0.96
Total antioxidants (mmol/l)	1.93^{b}	2.14^{a}	2.09^{a}	0.033	0.02

Means followed by different superscripts (a and b) within the same row are significantly different (P≤0.05).
G1: Control, G2: Herbal mixture and G3: Herbal mixture with yeast. SE (standard error). GPT: glutamic pyruvic transaminase. GOT: glutamic oxaloacetic transaminase.

Immunity status (IgM) was significant and higher in G2 and G3 than control. Meanwhile, there was a numerical increase of IgG in G2 and G3 compared to G1. These findings are in harmony with positive effect of herbal mixture in serum immunity status (Amagase *et al*., 2001; Redoy *et al*., 2020; Rabee *et al*., 2025). Moreover, oligosaccharides and β-glucans that are found in yeast (*Saccharomyces cerevisiae*) have an immunostimulant effect (Ghazanfar *et al*., 2017). Enhancement of immunity status is correlated with low susceptibility of diseases (Paul and Dey, 2015) and that may be decreased antibiotic use at the farm level. Total antioxidant concentrations were significant and higher in G2 and G3 than control by 9.59% and that may be due to the high antioxidant activity of herbal as (DPPH) scavenging activity that reached 41.38% at present study. These findings were in the same way that ruminant blood total antioxidant increased with herbal mixture and yeast supplementation (Kewan *et al*., 2021; Zhang *et al*., 2022).

3.6. Growth rate and feed conversion

Data of weight gain, growth rate and feed conversion are shown in Table 6 and Figure 1. Final body weight (FBW), total weight gain (TWG) and growth rate (GR) in the herbal mixture with yeast group (G3) were significant (P<0.05) and higher on average by 8.51, 13.50 and 13.50% than the other experimental groups, respectively. As shown in Figure 1, body weight increased similarly in G2 and G3 until reached the fourth month after that G3 was the highest and the control group was the lowest during the whole period.

That can be explained by higher values of nutrients digestibility and nutritive value (TDN, DCP, DE, ME) in G3 than the other groups (Tables 2 and 3). Also, the G3 group had a higher concentration energy supply as TVFAs (Table 4) and the higher concentration of thyroid hormones (T4 and T3) as shown Table 5. Additionally, phytochemicals in herbal mixture had a negative effect on ruminant internal parasite egg count and that may be increased growth rate (Raabe *et al*., 2025). Higher nutrient digestibility, nutritive value and growth rate were reflected on the lower feed conversion (higher feed efficiency) in the G3 than in the other groups. Kewan *et al*. (2021) reported that Barki sheep fed garlic and mixture of garlic and yeast had a higher growth rate and lower feed conversion than the control.

Table 6: Effect of herbal mixture (HM) and HM with yeast on final body weight, weight gain, growth rate and feed conversion of Barki lambs.

Item	G1	G2	G3	±SE	P value
IBW (kg)	13.72	13.25	13.43	0.90	0.82
FBW(kg)	37.63^{b}	38.22^{b}	41.15^{a}	0.52	0.01
TWG(kg)	23.91^{b}	24.97^{b}	27.72^{a}	0.59	0.02
GR (g/d)	103.96^{b}	108.58^{b}	120.53^{a}	2.55	0.02
Feed intake (kg)					
CFM	0.605	0.614	0.643	----	----
Hay	0.387	0.388	0.407	----	----
Total feed intake	0.992	1.001	1.050	----	----
Feed conversion					
DMI(kg)/1kg gain	9.54	9.22	8.71	----	----
TDNI(kg)/1kg gain	6.53	6.32	6.30	----	----
DCPI(kg)/1kg gain	1.07	1.02	1.03	----	----

Means followed by different superscripts (a and b) within the same row are significantly different (P≤0.05).
G1: Control, G2: Herbal mixture and G3: Herbal mixture with yeast. SE (standard error). IBW: initial body weight, FBW: final body weight, TWG: total weight gain, GR: growth rate, CFM: concentrate feed mixture. DMI: dry matter intake. TDN: total digestible nutrients. DCP: digestible crude protein.

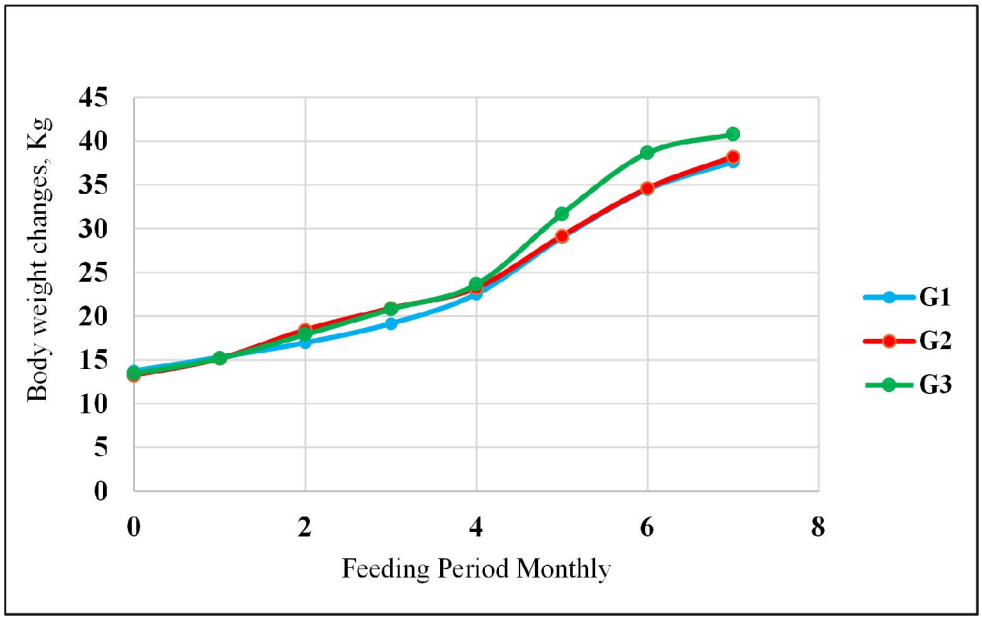

Fig. 1: Body weight changes of Barki lambs during experimental period fed on herbal mixture with or without yeast.

3.7. Carcass characteristics and meat color

Data of carcass characteristics of different experimental groups of Barki lambs are shown in Table 7 and 8. Dressing percent was non-significant and very close among all the groups, and the mean value equaled 47.70%. Carcass chilling loss was significantly (P<0.05) the lowest in the G3 compared to the other groups, which resulted in a higher cold carcass weight in the G3 group than the other groups by 5.52%. Low carcass chilling loss in G3 may be due to the high body weight, hot carcass weight and internal fat weight (Vaz *et al*., 2025). Low chilling loss means high cold carcass yield so, more revenue will be achieved from sold meat. These results were confirmed by Ghandour (2015) who reported that Barki sheep with higher hot carcass weight produced higher cold carcass and lower chilling loss (1.25%) versus the lower hot carcass weight which produced lower carcass weight and higher chilling loss (3.4%).

Table 7: Effect of herbal mixture (HM) and HM with yeast on dressing % and non-carcass components of Barki lambs.

Item	G1	G2	G3	±SE	P value
Fasting weight (kg)	35.53	36.23	37.60	1.43	0.850
Hot carcass weight (kg)	17.25	17.18	17.88	0.77	0.930
Cold carcass weight (kg)	16.77	16.71	17.68	0.76	0.860
Dressing (%)	48.43	47.16	47.51	0.39	0.430
Carcass chilling loss (%)	2.82[a]	2.77[a]	1.13[b]	0.24	0.002
Non carcass components					
Head (kg)	2.38	2.62	2.45	0.10	0.540
Pelt (kg)	4.00	3.67	4.35	0.23	0.510
Feet (kg)	0.89	0.88	0.90	0.03	0.920
Lung and trachea (g)	580.0	557.0	558.75	33.19	0.960
Liver (g)	452.0	557.0	573.75	28.80	0.180
Heart (g)	126.0	130.0	140.0	4.55	0.470
Kidney (g)	108.0	109.0	110.0	3.02	0.970
Spleen (g)	52.0	44.0	58.75	3.17	0.170
Testes (g)	229.0	286.0	285.0	20.99	0.480
Renal fat (g)	107.0	94.0	126.25	10.60	0.490
Abdominal fat (g)	227.0	206.0	367.50	36.99	0.150

Means followed by different superscripts (a and b) within the same row are significantly different (P≤0.05).
G1: Control, G2: Herbal mixture and G3: Herbal mixture with yeast. SE (standard error).

Although, non-carcass components were non-significant in all groups (Table 7) but there was a positive trend to reach higher values in the (G3) group such as in liver (573.75 g) and abdominal fat (367.50 g) than other groups and these may be due to high hot carcass weight in the G3 group. In the same way, Zayed *et al*. (2022) found related results on Barki sheep. The data of whole seal cuts, best ribs and meat color are shown in Table 8. The wholesale cuts values were non-significant differences among the groups. Neck, shoulders, lion, and tail were higher in the G1 group, only flank was higher in the G2 group, and racks and legs were higher in the G3 group. Also, the best ribs components values were non-significant among the groups, and their percentage distribution was as follows: lean and bone ratio were higher in the G2 group, while the fat was higher in the control group.

Meat color values were significantly (P<0.05) affected by dietary treatments (herbal mixture and probiotic). Herbal mixture group (G2) exhibited significantly (P < 0.05) the highest lightness (L*) and yellowness (b*), indicating a brighter and more yellow shifted color profile. Meanwhile, herbal mixture with probiotic group (G3) produced significantly (P <0.05) darker meat with lower lightness (L*) and yellowness (b*). Redness (a*) did not differ among treatments (P > 0.05). These results suggest that the G2 group diet improved color brightness and yellowness, whereas G3 group favored darker, and slightly more intense red coloration.

Moreover, Zeng and Shen (2025) reported that higher values of L*, a*, and b* in goats supplemented with phytogenic (garlic skin) compared to the control group. In the same pattern, Rabee *et al*. (2025) observed higher yellowness of meat color in goats that were fed with a herbal mixture (containing garlic and turmeric) than the control group. The source of the yellow color in the herbal mixture in the present study is mainly from turmeric. The yellow color (b) in turmeric reached a value equal to 69 as recorded by Mancini *et al*. (2015). Although the herbal mixture with the yeast group (G3) had the same herbal mixture daily but with yeast, the yellowness meat color (b*) was lower than group G2 by 85.67%, which may be explained by the high adsorption capacity of the yeast cell wall to different substances like plant pigment and mycotoxins (Echeverrigaray *et al*., 2020; Solovyov *et al*., 2020).

Table 8: Effect of herbal mixture (HM) and HM with yeast on carcass whole seal cuts, best ribs and meat color of Barki lambs.

Item	G1	G2	G3	±SE	P value
Wholesale cuts (% of cold carcass)					
Neck	6.22	5.77	5.84	0.20	0.560
Shoulders	20.34	20.17	19.20	0.26	0.160
Racks	24.89	25.70	26.16	0.30	0.240
Flank	3.49	3.94	3.07	0.16	0.070
Lion	6.93	6.64	6.70	0.21	0.860
Legs	33.42	33.15	34.53	0.43	0.400
Tail	4.71	4.62	4.49	0.31	0.970
Best ribs (9–10–11) (%)					
Lean	52.92	53.31	51.94	1.59	0.950
Bone	21.04	25.22	22.38	1.26	0.410
Fat	26.04	21.47	25.68	1.37	0.340
Eye muscle area (cm)^2	15.81	14.97	14.05	0.54	0.440
Meat color					
lightness (L)	42.16^a	46.28^a	39.52^b	1.12	0.030
Redness (a)	14.23	14.06	14.33	0.36	0.960
Yellowness (b)	7.47^b	12.18^a	6.56^b	0.81	0.002

Means followed by different superscripts (a and b) within the same row are significantly different (P≤0.05).
G1: Control, G2: Herbal mixture and G3: Herbal mixture with yeast. SE (standard error).

3.8. Histology

Microscopic examination of eye muscle tissue showed normal appearance of muscle tissue in all experimental groups (Fig. 2).

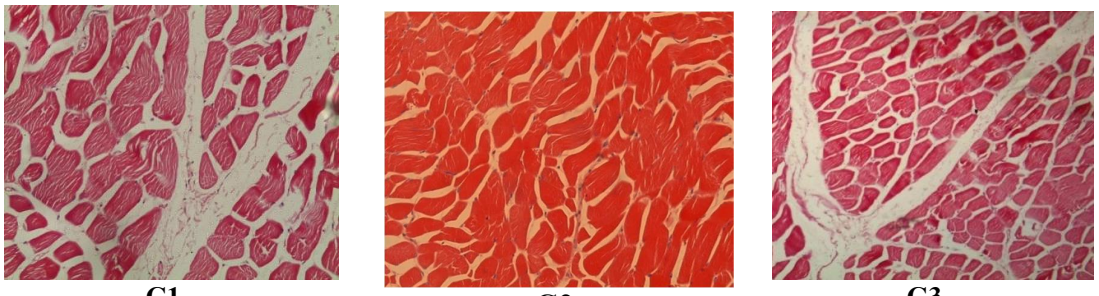

Fig. 2: The eye muscle histology of Barki lambs as affected by herbal mixture (HM) and HM with yeast compared to control group (H&E, X20).

This may be due to the blood metabolites of all experimental groups were in the normal range of healthy sheep (Table 5). Similar results were reported by Rabee *et al.* (2025) who found normal structure of eye muscle in both two groups of goats fed on control ration and herbal mixture.

The beneficial effects of herbal mixture with probiotic yeast supplementation on ruminant performance were confirmed by the results of the present study and previous studies. But the main constraint of herbal mixture and probiotic yeast supplementation to be applied at the farm level is the high cost of these feed additives. So, it could be highly recommended to use low price alternatives such as herbal mixture byproducts (garlic skin, leaves and straw) and highly efficient yeast produced in affordable growth media (Liao *et al*., 2022; Azzaz *et al*., 2024; Rabbani *et al*., 2024 ; Zeng and Shen 2025).

4. Conclusion

In conclusion adding probiotics to the herbal mixture enhanced feed digestibility, growth rate, immunity and carcass characteristics, and also decreased predicted methane intensity per one kilogram of body weight gain. These findings support improved sheep productivity and sustainability. Future studies on low-cost herbal mixture and probiotics are recommended.

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