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Effect Of Using Some Amino Acids On Gene Expression And Poultry Production

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ABSTRACT:
As a component of the Animal Production Research Institute (APRI), Egypt, the current study was conducted at the Animal Production Research Station in Sakha Kafr El-Sheikh. The purpose of this study was to assess the effects of supplementing local chicken breed AL-SALAM with dietary methionine or lysine amino acids on growth performance, feeding, dressing percentage of carcass, serum chemistry and RNA expression levels of various tissues including the liver, intestine, and breast muscle (Atrogene-1, IGF-1, GAL6, and MUC2 genes). The results showed a daily increase in body weight, carcass yield, lysozyme activity, conversion rate, decreased feed consumption and a change in the expression of mRNA (Atrogin-1, IGF-1, genes in breast muscle, MUC2 in the intestine and GAL6 in the liver) in comparison to the control group. The levels of methionine and lysine supplementation were 0.50, 0.75, and 1 g methionine and 1.50, 2, and 2.50 g lysine respectively. When access amino acid levels were added, relative economic efficiency outperformed the control group. We are suggestion that adding access methionine with level 0.5% or lysine with level 2.5% to reach market weight.

INTRODUCTION:

Nutritionally, amino acids are divided into two categories: essential and non-essential. Amino acids are the structural and functional building blocks of protein.The body uses amino acids for essential physiological functions (**Debnath et al., 2019**). Maintaining the health of pullets, laying hens' ability to produce high-quality eggs and broiler body growth all depend heavily on nutrition (**Wang et al., 2017**). Common components of chicken rations or a mixture of them (**Horvath and Babinsky 2018**) such as vitamins (**Ahmad et al., 2019**), minerals (**Khatun et al., 2019**) and amino acids (**Ghoreyshi et al., 2019**) can be nutraceuticals, particularly crucial in poultry feeding.Methionine regarded as the broiler's first limiting amino acid. It contributes significantly to the optimal growth performance of chicken and is engaged in muscle accretion, feather synthesis, and critical biochemical activities (as a methyl group donator) (**Fagundes et al., 2020**).**Abdallah et al.** (**2022**) showed that, supplementing the local chicken breed diet with the amino acids methionine or lysine increased daily body weight, decreased daily feed intake, increased of carcass dressing percentage and decreased feed consumption.The objectives of the present study to improve Egyptian breed chicken for growth performance, meat yield and changes in the RNA expression levels of different tissues breast muscle, liver and intestine (Atrogene-1, IGF-1, GAL6 and MUC2 genes) in terms of methionine or lysine supplementation of feed.

MATERIALS AND METHODS

The current study was conducted at the Animal Production Research Institute's (APRI) Animal Production Research Station in Sakha Kafr El-Sheikh, Egypt. The biochemical examination was conducted over the summer months of June through September 2023 in APRI's laboratories.

Experimental Birds and Management:

A total of four hundred and twenty (420) 14-day-old unsexed, local broiler chicks of the AL-Salam breed were randomized to seven experimental diets. In a fully randomized design, 60 birds (60 chickens per treatment) were used to each treatment.Each treatment was further split into three replicates, each of which had 20 chickens. The chickens were raised in deep litter with unlimited access to food and water. The average weight of each chick was 30.93±0.14 g./bird.Chickens were vaccinated against Marek's, Newcastle, infectious bronchitis and Bursal diseases. The experimental period started from 1week up to 16 weeks old chicks.

The experiment was conducted to investigate the effects of amino acids (Methionine and/ or Lysine) powder to broiler chickens' diet on growth performance, feeding, dressing percentage of carcass, serum chemistry and RNA expression levels.Seven experimental groups, three levels for each, there were tested comparing to control group (C) feeding on commercial diet basis in **(Table 1).** Firstly, methionine amino acid levels in three groups (M1, M2 and M3) were supplemented with 0.50 %, 0.75 % and 1.00 % of feed respectively. Secondly, lysine amino acid levels groups (L1, L2 and L3) were supplemented with 1.50 %, 2.00 % and 2.50 % of chicken feed.

Table (1): Chemical composition of basic diet:

Chemical analysis (on DM basis, %)	Control diet
Dry matter (%)	88
Crude protein	18.7
Crude fiber	3.35
Ether extract	5.7
Digestible energy (kcal/ kg)	3107
Methionine and or Lysine	0.2%

Experimental Diet:

Chicks were fed starter diets at the first two weeks and afterward they were placed on finisher experimental diets. The control diet contained 0.20 % amino acids while the other six diets contained 0.50, 0.75 and 1.00 % methionine groups (M1, M2 and M3 levels while lysine groups diets were 1.5, 2.00 and 2.5 % lysine levels for L4, L5 and L6 feeding respectively, in **(Table 2)**.

Table 2: Amino acids lysine and /or methionine composition (g /Kg) of control and experimental diets.

Groups	Treatments
Basic diet (Control) C	0.2% methionine and/or 0.2% lysine
Methionine M1	0.50 %
Methionine M2	0.75 %
Methionine M3	1.00 %
Lysine L1	1.50 %
Lysine L2	2.00 %
Lysine L3	2.50 %

In chickens, the feed conversion ratio is influenced by management practices, age, feed quality, and genetics. Commercial poultry feed meal served as the base diet. The results were analysed using Statistical Analysis Software **(SAS, 2013)** and folding change of gene expression in high temperature during the summer season. Body weight gain (BWG) was measured weekly (BWG = BW2 – BW1), and average weight gain was calculated every 4 weeks. Daily feed intake, final body weight, and carcass percentage % were evaluated for 9 chicks from each treatment at the end of the experimental period.

Sample collection:

Three birds per group were chosen at random at the conclusion of the experiment, weighed, and then killed. Feed intake (FI), body weight growth (BWG), feed conversion ratio (FCR), and initial and final live body weight (IBW and FBW) were all noted. Nine chicks from each treatment had their carcass features assessed at the conclusion of the trial.

Feed Intake (FI):

Feed intake was determined weekly and average FI was calculated every 4 weeks. Feed intake means determined as daily feed intake (DFI).Total feed intake in 4 weeks period / number of chicks = average feed intake/bird/4 weeks, and then divided on 28 days to reach mean feed intake/bird/day.

Feed Conversion Ratio (FCR):

FCR was measured weekly and average FCR was calculated every 4 weeks. Feed conversion ratio means calculated as daily feed conversion ratio. Total feed conversion in 4 weeks period / number of chicks = average feed conversion/bird/4 weeks, and then divided on 28 days to reach average feed conversion ratio/bird/day.

Dressing percentage of carcass %:

Data collection at the end of the experimental periods three birds from each replicate of each dietary treatment were randomly selected and weighed individually, then slaughtered and allowed to bleed.

Dressing percentage, non-carcass component (heart, gizzard, liver, giblets and spleen) was weighed and means were calculated.

Gene expression:

For gene expression analysis, samples of liver, intestines and breast muscles were collected from each treatment for local breed El-Salam chickens after slaughtered at 16 weeks old.

a) RNA extraction:

Liquid nitrogen reagent was used for RNA extraction according to manufacturer recommendations, 1mL/100 mg tissue. The RNase inhibitors, Thermo RNase gene jet RNA purification Kits, were used to prepare laboratory materials.

b) Gel Electrophoresis:

It was evaluated on 1% agarose gel stained with 10% ethidium bromide and observed using ultraviolet light.

c) cDNA Synthesis:

Thermo Scientific Maxima First Strand cDNA Synthesis Kit for RT-qPCR #K1641 The Maxima® First Strand cDNA Synthesis Kit is a convenient system optimized for cDNA synthesis in two step real time quantitative RT-PCR (RT-qPCR) applications (**Table 3**).

Real Time-PCR:

Real-time polymerase chain reaction (RT-PCR) of liver, intestine and breast muscle samples were performed in National bio lab, Giza, Egypt using Fluorescent dye SYBR Green (SYBR® Green PCR Master Mix (Applied Bio systems).

Table (3): The qRT-PCR primers used in this study. insulin-like growth factor-I (IGF-I); muscle atrophy-induced ubiquitin ligases (atrogin-1); Gallinaceans (GAL6) and Mucin2 (MUC2). (http://www. ncbi.nlm.nih.gov/genbank/).

Gene	Temperature	Primer sequence (5'-3')	Product size bp
IGF-I	60°C	CATTTCTTCTACCTTGGC TCATCCACTATTCCCTTG	260
GAL6	60°C	CTCTTCCAGGCTGCTCCAGCTTAC TTAGGAGCTAGGTGCCCATTTG	312
Atrogin-1	60°C	ACTTTGGTTCAACGGGTCG CGGTCTTCGCTGAGCACTT	119
MUC2	60°C	TCACCCTGCATGGATACTTGCTCA TGTCCATCTGCCTGAATCACAGGT	69
B- Actin	60°C	TGCTGTGTTCCCATCTATCG TTGGTGACAATACCGTGTTCA	136

Statistical analysis:

All data were subjected to one-way statistical analysis according to (**SAS, 2013**).

The differences among groups means were determined according to Duncan's multiple rang test (**Duncan, 1955**). Regarding the gene expression statistical analysis, the following equations have been used:

£ΔCt = Ct (a target gene) – Ct (housekeeping gene).

The ΔCt where used to calculate ΔΔCt according to following equation:

ΔΔCt = ΔCt (treated sample) – ΔCt (untreated sample) and from this knowledge the fold of gene expression change calculated by as $2^{-\Delta\Delta Ct}$.

RESULTS AND DISCUSSION

The effect of Methionine or Lysine supplemented diets on El-Salam chicken:

The present study confirmed that methionine and/or lysine essential amino acids supplementation significantly improved El-Salam chicken local breed weight. The best results obtained by the chicks fed the methionine and lysine levels were (0.5% and/or 2.5%), respectively for performance live body weight, immunity increase, carcass % and most genes expressions. In addition, the body weight of birds at 16 weeks ages were obtained in **Table (4)** indicates that, as expected, there were statistically significantly at ($P \leq 0.05$). The present study confirmed that methionine/or lysine amino acids supplementation significantly improved bird's weight. Data for daily body weight gain (DBWG), final body weight (FBW) at 16 weeks of the end experimental, daily feed intake (DFI), feed conversion rate (FCR), carcass yield (dressing percentage of carcass %) during summer season (high temperature).The results presented in **Table (4)** regarding, the daily body weight gain (DBWG) from start to final experimental period (0-16 weeks of age) as results were (11.29± 0.03 g) by M1 (0.5% methionine/weight of diet) in methionine groups and (10.70±0.35 g) by L3 (2.50 % lysine/weight of diet) in lysine groups respectively. These results showed a significant increase in the DBWG and FBW (g) at 16 weeks of age by treatments at ($P \leq 0.05$) compared with control group and other treatment groups **Figure (1).** Our findings agreed with those of **Ahmed and Abbas (2011)** who found that broiler body weight gain and breast muscle weight were increased when feeding methionine levels exceeded **NRC (1994)** in comparison to the control feed. Additionally, it was suggested that the increased growth performance of chickens was due to the fact that methionine in the meals increases the hens' ability to absorb and digest nutrients by increasing the villus's length and height **(Lee et al., 2020).**

Table (4): Effect of dietary amino acids methionine or lysine on daily body weight gain means (DBWG g) during Experimental period from (0 - 16 weeks old chicks).

Treatment	Initial body weight(g)	Final body weight(g)	DBW(g) 0-4	DBW(g) 4-8	DBW(g) 8-12	DBW(g) 12-16	DBW(g) 0-16
Control C	30.93 ± 0.14^{ab}	1237.13 ± 6.55^{b}	6.89 ± 0.34^{a}	9.64 ± 0.56^{a}	11.11 ± 0.97^{b}	13.76 ± 0.09^{a}	10.35 ± 0.34^{c}
M1	31.97 ± 0.14^{a}	1405.50 ± 3.38^{a}	7.08 ± 0.58^{a}	9.87 ± 0.27^{a}	13.56 ± 2.46^{a}	14.94 ± 1.98^{a}	11.29 ± 0.03^{a}
M2	30.78 ± 0.08^{ab}	1367.16 ± 4.34^{b}	7.76 ± 0.07^{a}	9.75 ± 0.33^{a}	12.50 ± 1.66^{ab}	13.25 ± 1.53^{a}	10.82 ± 0.38^{b}
M3	31.18 ± 0.10^{ab}	1388.13 ± 3.57^{b}	7.05 ± 0.19^{a}	9.78 ± 0.91^{a}	12.46 ± 1.092^{ab}	13.69 ± 0.66^{a}	10.75 ± 0.08^{ab}
L1	28.60 ± 2.13^{b}	1319.4 ± 2.72^{c}	6.88 ± 0.82^{a}	8.92 ± 1.40^{a}	13.13 ± 0.88^{a}	13.65 ± 1.10^{a}	10.65 ± 0.35^{ab}
L2	31.09 ± 0.14^{a}	1342.0 ± 3.57^{b}	6.58 ± 0.59^{a}	8.83 ± 1.00^{a}	11.31 ± 0.98^{b}	13.79 ± 3.66^{a}	10.13 ± 0.66^{abc}
L3	31.38 ± 0.08^{a}	1358.7 ± 4.34^{b}	6.98 ± 0.60^{a}	9.02 ± 1.08^{a}	12.89 ± 1.17^{ab}	13.89 ± 1.64^{a}	10.70 ± 0.60^{bc}
P value	0.24	0.95	0.98	0.38	0.06	0.68	0.03

a, b, and c: Means ±S.E. denoted on each column with different superscripts are significantly different at ($P \leq 0.05$). C: control group feed on basal feeding, M1: group1 supplemented with 0.5% methionine, M2: group 2 supplemented with 0.75% methionine, M3: group 3 supplemented with 1% methionine, L1: group 4 supplemented with 1.5% lysine, L2: group 5 supplemented with 2% lysine and L3: group 6 supplemented with 2.5% lysine levels of feed**.**

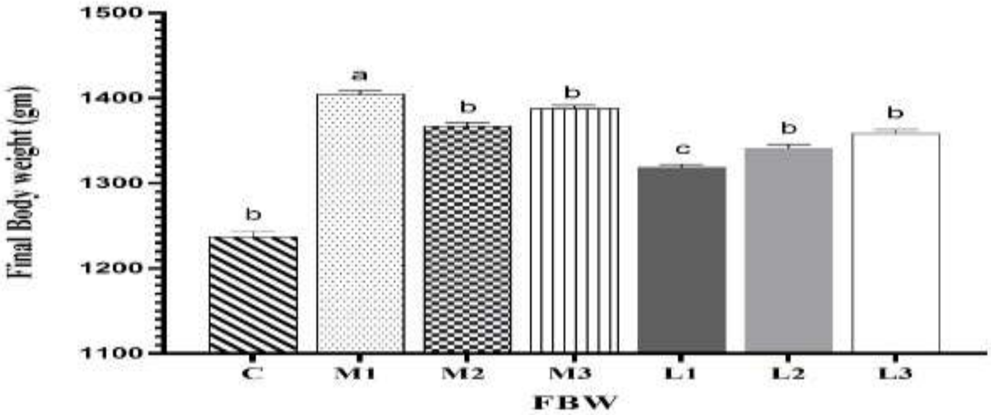

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Figure (1): Effect of methionine and lysine levels on final body weight (FBW) of El-Salam Chickens breed at 16 weeks age.

a, b and c: Means ± S.E. denoted on each column with different superscripts are significantly different at (P ≤ 0.05). C: control group feed on basal feeding, M1: group1 supplemented with 0.50% methionine, M2: group 2 supplemented with 0.75% methionine, M3: group 3 supplemented with 1.00.% methionine, L1: group 4 supplemented with 1.50% lysine, L2: group 5 supplemented with 2.00% lysine and L3: group 6 supplemented with 2.50% lysine levels of feed.

The results showed that, second group (M1) and seventh group (L3), there were significantly increased of feed consumption at (P≤0.05) with the increase of methionine diet supplementation levels for daily feed intake (DFI) at (4-8), (12-16) and (0-16) weeks of experimental period compared with control group (C) in (**Table 5 and Figure 2**).

Table (5): Effect of dietary amino acids methionine or lysine on daily feed intake (DFI) during the experimental period (0 - 16 Weeks old chicks).

Treatments	DFI 0-4 weeks (g)	DFI 4-8 weeks (g)	DFI 8-12 weeks (g)	DFI 12-16 weeks (g)	DFI 0-16 weeks (g)
Control C	20.12±0.23b	40.29±0.16^{b}	63.87±0.23^{c}	79.71 ±0.44^{c}	50.98±0.18^{c}
M1	20.75±0.35^{a}	42.20±0.23^{a}	77.48±0.35^{a}	85.87±0.35^{a}	53.16±0.22^{a}
M2	20.69±0.32^{a}	41.32±0.22^{b}	64.14±0.34^{b}	79.68±0.34^{c}	51.83±0.80^{b}
M3	20.13±0.16^{b}	41.43±0.12^{b}	64.06±0.25^{bc}	79.75±0.45^{c}	52.89±0.23^{b}
L1	20.43±0.22^{b}	41.67±0.19^{b}	63.16±0.43^{c}	79.73±0.36^{c}	51.33±0.34^{c}
L2	20.75±0.06^{a}	41.38±0.13^{b}	64.57±0.22^{bc}	79.94±0.38^{b}	52.16±0.24^{b}
L3	20.24±0.21^{b}	42.15±0.11^{a}	70.21±0.33^{a}	81.02±0.36^{b}	54.96±0.25^{a}
P value	0.31	0.001	0.02	0.05	0.002

a, b and c: Means ± S.E. denoted on each column with different superscripts are significantly different at (P ≤ 0.05). C: control group feed on basal feeding, M1: group1 supplemented with 0.50% methionine, M2: group 2 supplemented with 0.75% methionine, M3: group 3 supplemented with 1.00.% methionine, L1: group 4 supplemented with 1.50% lysine, L2: group 5 supplemented with 2.00% lysine and L3: group 6 supplemented with 2.50% lysine levels of feed.

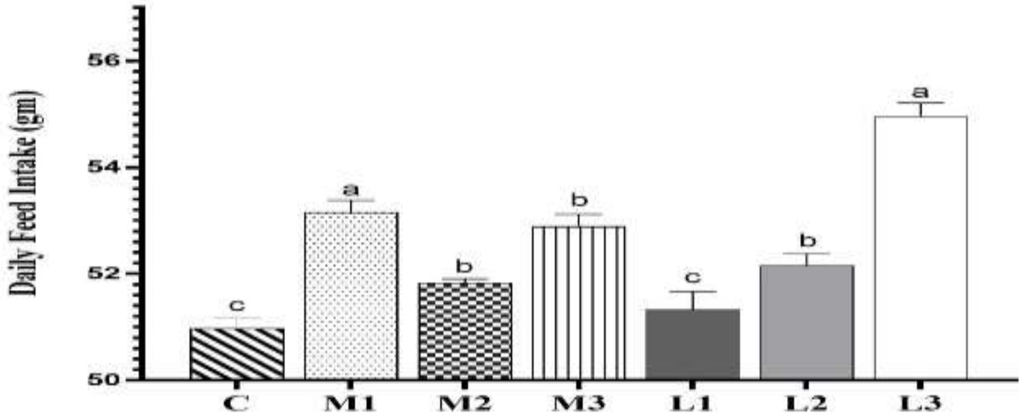


Figure (2): Average of daily feed intake (DFI) for El-Salam chicken fed control and three levels of Methionine and lysine diets from 0-16 weeks.

a, b and c: Means ± S.E. denoted on each column with different superscripts are significantly different at (P ≤ 0.05). C: control group feed on basal feeding, M1: group1 supplemented with 0.50% methionine, M2: group 2 supplemented with 0.75% methionine, M3: group 3 supplemented with 1.00.% methionine, L1: group 4 supplemented with 1.50% lysine, L2: group 5 supplemented with 2.00% lysine and L3: group 6 supplemented with 2.50% lysine levels of feed.Feed intakes were better for groups M1 and L3 which fed 0.5% methionine or 2.50 % lysine levels of diet for native poultry. **Lee et al., (2020)** showed that chicken development performance was improved because methionine in the meals helps hens' boosts their capacities to absorb and digest nutrients in the foods. Methionine is directly utilized by animals as a precursor for protein synthesis **(Fang et al., 2010)**. The findings shown in Table 6 and Figure 3 are consistent with **Cruz et al. (2017)** who demonstrated that increasing the dietary Lys. Level significantly raised growth performance levels and FCR in every instance. However, these findings concurred with those of **Chai et al. (2020)** who found that lowering lysine in a low-crude-protein diet resulted in the lowest levels of BWG, FI, and GR. Feed was effectively transformed into live weight, according to the FCR.

Table (6): Effect of dietary amino acids methionine or lysine on feed conversion rate (FCR) during the

Experimental period. (0- 16 Weeks old chicks).

Treatments	FCR 0-4 weeks (g)	FCR 4-8 weeks (g)	FCR 8-12 weeks (g)	FCR 12-16 weeks (g)	FCR 0-16 weeks (g)
Control C	4.17±0.31^{a}	5.54±0.76^{a}	6.89±0.88^{a}	11.68±0.17^{a}	6.34±0.07^{a}
M1	3.49±0.35^{c}	4.83±0.31^{b}	4.24±0.74^{b}	9.10±1.94^{b}	5.43±0.03^{c}
M2	4.27±0.10^{b}	4.88±0.19^{b}	5.34±0.63^{b}	9.15±1.66^{b}	5.75±0.31^{c}
M3	4.29±0.17^{b}	4.94±0.58^{b}	5.70±0.56^{b}	10.56±0.91^{ab}	5.90±0.25^{b}
L1	4.42±0.71^{b}	5.06±0.18^{b}	4.27±0.23^{b}	11.82±1.64^{a}	6.04±0.43^{b}
L2	3.72±0.43^{c}	5.00±0.66^{b}	5.49±0.55^{ab}	9.52±1.51^{b}	6.03±0.41^{b}
L3	3.56±0.35^{c}	4.96±0.94^{b}	4.77±0.84^{b}	9.09±2.57^{b}	5.74±0.23^{c}
P value	0.41	0.89	0.18	0.94	0.61

a, b and c: Means ± S.E. denoted on each column with different superscripts are significantly different at (P ≤ 0.05). C: control group feed on basal feeding, M1: group1 supplemented with 0.50% methionine, M2: group 2 supplemented with 0.75% methionine, M3: group 3 supplemented with 1.00.% methionine, L1: group 4 supplemented with 1.50% lysine, L2: group 5 supplemented with 2.00% lysine and L3: group 6 supplemented with 2.50% lysine levels of feed.

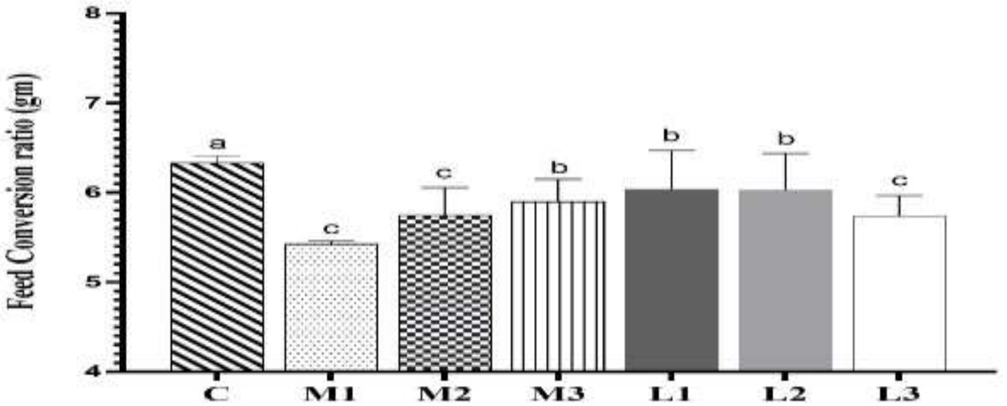


Figure (3): Means ±S.E. denoted on each column with different superscripts are significantly different at ($P \leq 0.05$). C: control group feed on basal feeding, M1: group1 supplemented with 0.50 % methionine, M2: group 2 supplemented with 0.75% methionine, M3: group 3 supplemented with 1.00 % methionine, L1: group 4 supplemented with 1.50 % lysine, L2: group 5 supplemented with 2.00% lysine and L3: group 6 supplemented with 2.50% lysine levels of feed**.**

2- Dressing percentage of carcass %:

From the presented data in (**Table 7**) and (**Figure 4**), it could be observed that Methionine and lysine groups (M1, M2, M3 and L1, L2, L3) had higher final body weight and carcass yield at end experimental period in comparison to the control group (C).

Table (7): Effect of dietary amino acids on carcass characteristics at the end of the experimental period (16 weeks old chicks).

Treatment	Final body weight (g)	Carcass %	Heart %	Liver%	Gizzard%	Giblets%	Spleen%
Control C	1237.13±6.55^{d}	70.19±0.80^{b}	0.47±0.03^{a}	3.44±0.78^{a}	1.44±0.21^{a}	5.35±0.92^{a}	0.49±0.16^{a}
M1	1405.50±3.38^{a}	73.25±1.03^{a}	0.42±0.03^{a}	3.48±0.20^{a}	1.36±0.14^{a}	5.18±0.29^{a}	0.38±0.05^{ab}
M2	1367.16±4.34^{b}	70.43±0.99^{b}	0.37±0.02^{a}	2.31±0.09^{b}	1.66±0.18^{a}	4.34±0.10^{a}	0.22±0.03^{b}
M3	1388.13±3.57^{b}	71.85±3.82^{b}	0.31±0.08^{a}	2.95±0.37^{b}	1.59±0.08^{a}	4.85±0.51^{a}	0.24±0.01^{b}
L1	1319.4±2.72^{c}	71.13±1.02^{b}	0.45±0.01^{a}	2.39±0.07^{b}	1.63±0.06^{a}	4.47±0.09^{a}	0.35±0.05^{ab}
L2	1342.0±3.57^{b}	72.74±0.19^{b}	0.43±0.08^{a}	3.31±0.45^{a}	1.71±0.17^{a}	4.32±0.36^{a}	0.26±0.03^{b}
L3	1358.7±4.34^{b}	73.84±0.16^{a}	0.48±0.11^{a}	2.55±0.03^{b}	1.65±0.15^{a}	4.68±0.07^{a}	0.24±0.02^{b}
P value	0.95	0.16	0.51	0.17	0.66	0.45	0.11

a, b and c: Means ± S.E. denoted on each column with different superscripts are significantly different at ($P \leq 0.05$). C: control group feed on basal feeding, M1: group1 supplemented with 0.50% methionine, M2: group 2 supplemented with 0.75% methionine, M3: group 3 supplemented with 1.00.% methionine, L1: group 4 supplemented with 1.50% lysine, L2: group 5 supplemented with 2.00% lysine and L3: group 6 supplemented with 2.50% lysine levels of feed.

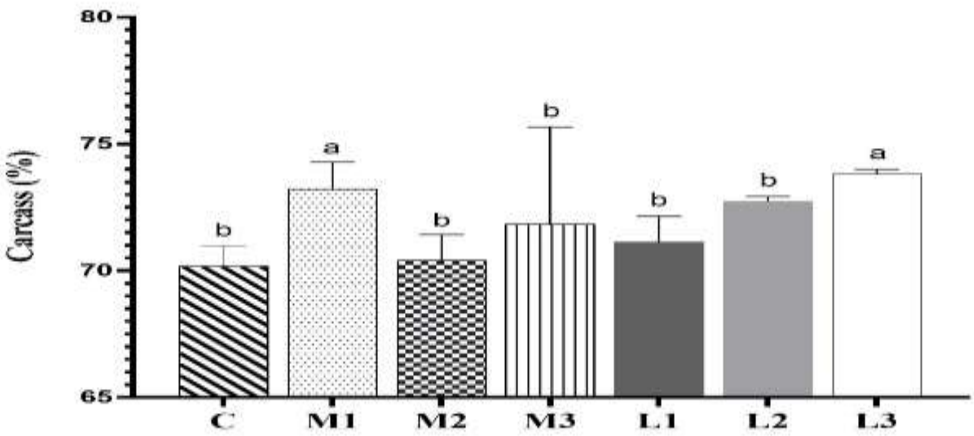


Figure (4): Carcass dressing percentage of El-Salam chicken fed control and three levels of Methionine diets at 16 Weeks.

a, b and c: Means ± S.E. denoted on each column with different superscripts are significantly different at (P ≤ 0.05). C: control group feed on basal feeding, M1: group1 supplemented with 0.50% methionine, M2: group 2 supplemented with 0.75% methionine, M3: group 3 supplemented with 1.00.% methionine, L1: group 4 supplemented with 1.50% lysine, L2: group 5 supplemented with 2.00% lysine and L3: group 6 supplemented with 2.50% lysine levels of feed.

According to **Linh et al. (2021)** 0.25% methionine supplementation raised carcass weight linearly (P≤0.05), which is in keeping with our findings. However, there was no discernible alteration in internal organs (P≤0.05). Methionine supplementation enhances chicken carcass characteristics, which is one feed additive that can impact meat quality **(Zhang et al., 2017).** Because chicken muscle and edible organs, where protein synthesis takes place are significantly impacted by the amino acid methionine **(Jariyahatthakij et al., 2018)**.

3- Gene Expression analysis:

Broilers fed methionine or lysine supplementation showed changes in the RNA expression levels of different tissues for breast muscle, liver and intestine (Atrogene-1, IGF-1, GAL6 and MUC2 genes).

a) Atrogin-1:

Table (8): Fold change estimation of Atrogin-1 gene by affected with methionine /or lysine levels

in Al-Salam feed **chicken** at 16 weeks age**.**

Sample	b-actin=B	Atrogin=A	Δ ct=A-B	Avg	ΔΔ ct	Avg. Fold chan
M1	26.39	28.77	2.38	2.51	-0.27	1.20
M1	27.36	30.11	2.75			
M1	26.72	29.11	2.39			
M2	25.19	25.43	0.24	1.77	-1.00	2.00
M2	22.52	27.20	4.68			
M2	27.00	27.40	0.40			
M3	23.85	27.22	3.37	2.24	-0.54	1.45
M3	26.46	27.01	0.55			
M3	24.54	27.33	2.79			
C	22.03	26.54	4.51	2.77	0	1.00
C	24.50	26.26	1.76			
C	25.65	27.70	2.05			
L1	23.57	26.88	3.31	2.26	-0.51	1.42
L1	22.01	26.44	4.43			
L1	27.23	26.28	-0.95			
L2	25.32	26.12	0.80	2.20	-0.57	1.49
L2	23.19	26.78	3.59			
L2	24.74	26.95	2.21			

L3	23.74	26.22	2.48	2.50	-0.28	1.21
L3	26.52	26.54	0.02			
L3	21.17	26.15	4.98			

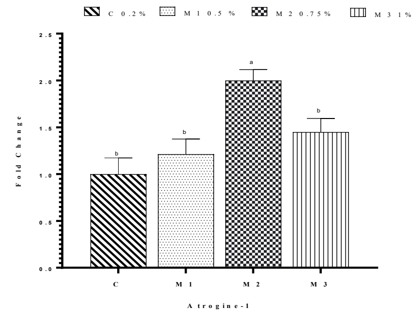


Figure (5a): Expression of Atrogene -1 gene in breast muscle Of El-Salam at different methionine levels.

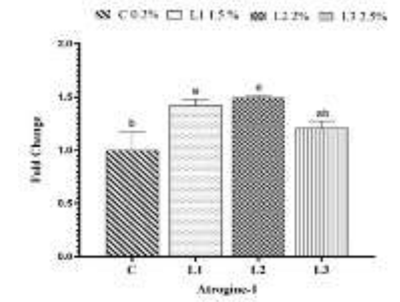


Figure (5b): Expression of Atrogine-1 gene in breast muscle Of El-Salam chicken at different lysine levels.

The results of the Atrogene-1 gene expression of the El-Salam chickens local Egyptian breed at 16 weeks old are shown in (**Figure5 a, b**), the mean of fold change for gene expression of Atrogene-1 was 2.00±0.12 this increase and significantly more than other groups, while control, M1and M3 non-significantly and recorded values 1±0.17, 1.20±0.16 and 1.45±0.14-fold change.Our results agreement with (**Mai et al., 2019**) who reported that, the expression of mRNA atrogine-1 and other genes in breast muscle and expression of mRNA PI3KR1 in liver are affected by methionine levels in diet.

b): IGF-1:

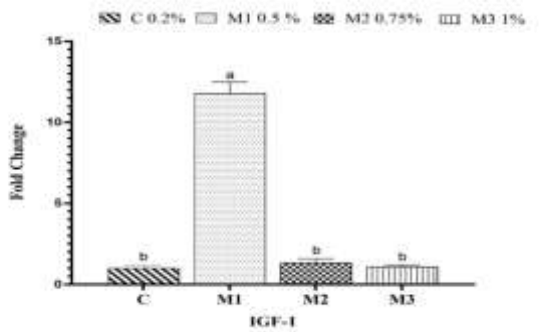
Protein deposition, which is controlled by the equilibrium between protein synthesis and breakdown, is primarily responsible for chicken growth. When the liver of chickens was tested for the expression of the gene for the antimicrobial peptide 2 (IGF-1), the results differed depending on the treatment group (**Figure 6a, b**).

Result of second group (M1) supplemented with 0.50% methionine in feed chicken in gene expression was increased significantly (P ≤ 0.05) higher than the control group (C) and other treatments groups.

Table (9): Fold change estimation of IGF-1 gene by affected with methionine /or lysine levels in Al-Salam feed chicken at 16 weeks age.

Sample	**b-actin=B**	**IGF-1=A**	**Δ ct=A-B**	**Avg.**	**ΔΔ ct**	**Avg. Fold change**
M1	26.39	25.10	-1.29	0.33	-356	11.77
M1	27.36	27.37	0.01			
M1	26.72	29.00	2.28			
M2	25.19	27.37	2.18	3.57	-0.32	1.25
M2	22.52	29.27	6.75			
M2	26.40	28.18	1.78			
M3	23.85	31.18	7.33	3.79	-0.10	1.07
M3	26.46	27.10	0.64			
M3	24.54	27.94	3.40			
C	22.03	29.24	7.21	3.89	0	1.00
C	24.50	30.52	6.02			
C	25.65	24.09	-1.56			
L1	23.57	2480	1.23	1.50	-2.39	5.25
L1	22.01	26.50	4.49			

L1	27.23	26.00	-1.23			
L2	25.32	24.90	-0.42	1.41	-2.48	5.58
L2	23.19	26.50	3.31			
L2	24.74	26.08	1.34			
L3	23.74	25.37	1.63	0.73	-3.16	8.94
L3	26.52	25.50	-1.02			
L3	21.17	22.75	1.58			



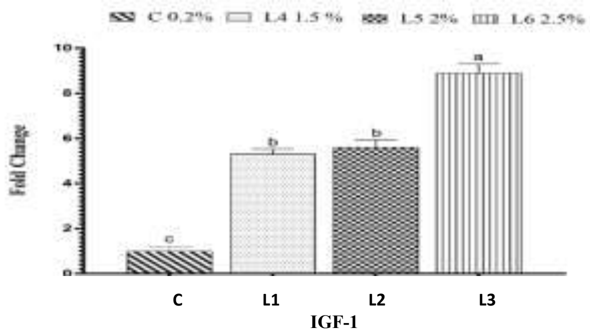


Figure (6a): Expression of IGF-1 gene in breast muscle chicken at different methionine levels.

Figure (6b): Expression of IGF-1 gene in breast muscle of El-Salam chicken at different lysine levels.

The fold changes were (11.77±0.70) in M1, (1.25±0.26) in M2 and (1.07±0.12) in M3 treatment groups, respectively but the value average of fold change of IGF-1 gene in control group was (1.00±0.2) **Table (9).**

This trail revealed highest amounts of IGF-1 mRNA and GHR mRNA, and best broiler growth performance in animals fed methionine supplementation **(Mai El-Tarras et al., 2019).**

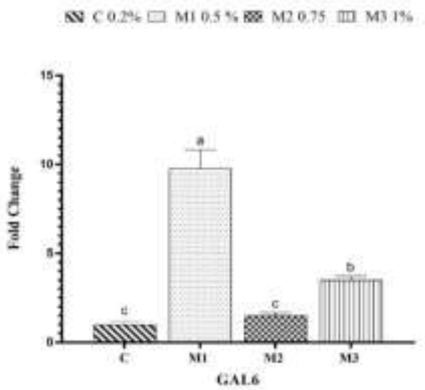
c) GAL6:

The GAL6 gene's expression in the liver and spleen was examined. The findings were examined and displayed as a bar graph in (**Table 10 and Figure 7a, b**) When compared to the control diet (C), GAL6 gene expression was generally considerably ($P < 0.05$) higher across all experimental diets.

Table (10): Fold change estimation of GAL6 gene by affected with methionine /or lysine levels in Al-Salam feed chicken at 16 weeks age.

Sample	b-actin=B	GAL6=A	Δ ct=A-B	Avg.	ΔΔ ct	Avg. Fold change
M1	26.39	27.63	1.24	1.67	-3.29	9.76
M1	27.36	30.96	3.60			
M1	26.72	26.90	0.18			
M2	25.19	27.42	2.23	4.39	-0.57	1.48
M2	22.52	31.49	8.97			
M2	26.40	28.37	1.97			
M3	23.85	26.22	2.37	3.14	-1.82	3.53
M3	26.46	31.13	4.67			
M3	24.54	26.92	2.38			
C	22.03	26.76	4.73	4.96	0	1.00
C	24.50	31.13	2.58			
C	25.65	33.22	7.57			

L1	23.57	24.19	0.62	2.13	-2.83	7.13
L1	22.01	26.00	3.99			
L1	27.23	29.00	1.77			
L2	25.32	29.78	4.46	2.18	-2.78	6.88
L2	23.19	25.00	1.81			
L2	24.74	25.00	0.26			
L3	23.74	26.00	2.26	1.82	-3.14	8.79
L3	26.52	24.90	-1.62			
L3	21.17	26.00	4.83			



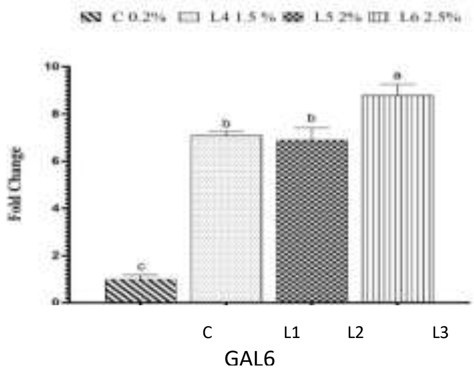


Figure (7 a): Expression of GAL6 gene in liver of El-Salam chicken fed control and three levels of methionine diets.

Figure 7b): Expression of GAL6 gene in liver of El-m chicken at different lysine levels.

The results in (**Table 10&Figures 7a&b**) showed the GAL6 gene expression was increase significantly at ($P \leq 0.05$) in seventh group (L3) feeding diet with 2.5% lysine supplemented than more control and other groups where recorded (8.8±0.46) in fold change.

The effect of high amino acids methionine or lysine in the diet of broilers was significant ($P \leq 0.05$) increase of GAL6 gene expression in the liver. The effect of increased of lysine of amino acid up to 2.5% of feed in chicken was significant ($P \leq 0.05$) increased of GAL6 gene expression in the liver. Also, results were non-significantly at ($P \leq 0.05$) between fifth (L1) and sixth (L2) groups values (7.13±0.17) and (6.88±0.52) values respectively, when feeding supplemented with lysine but were increased significantly compared with control group (C).

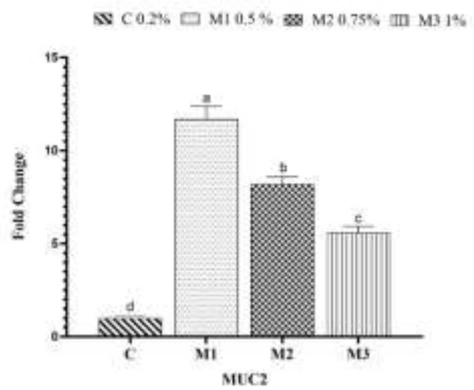
D): MUC2

The MUC2 gene's expression in the small intestine's jejunum was examined. When compared to the control diet (C), the MUC2 gene expression was considerably ($P \leq 0.05$) higher across all experimental diets, according to the results, which were evaluated and displayed in a bar graph as shown in (**Table 11 & Figures 8a,b**)

Table (11): Fold change estimation of MUC2 gene by affected with methionine /or lysine levels in Al-Salam feed chicken at 16 weeks age.

Sample	b-actin=B	MUC2=A	Δ ct=A-B	Avg.	ΔΔ ct	Avg. Fold change
M1	26.39	26.66	0.27	1.63	-3.54	11.66
M1	27.36	32.10	4.74			
M1	26.72	26.60	-0.12			
M2	25.19	27.00	1.81	2.14	-3.04	8.21
M2	22.52	25.52	3.00			
M2	26.40	28.00	1.60			
M3	23.85	27.50	3.65	2.68	-2.49	5.62

M3	26.46	28.00	1.54			
M3	24.54	27.40	2.86			
C	22.03	29.83	7.80	5.17	0.00	1.00
C	24.50	26.76	2.26			
C	25.65	31.11	5.46			
L1	23.57	26.70	3.13	2.91	-2.26	4.79
L1	22.01	27.09	5.08			
L1	27.23	27.76	0.53			
L2	25.32	27.40	2.08	2.08	-3.09	8.51
L2	23.19	25.60	2.41			
L2	24.74	26.50	1.76			
L3	23.74	25.00	1.26	1.70	-3.47	11.08
L3	26.52	28.53	2.01			
L3	21.17	23.01	1.84			



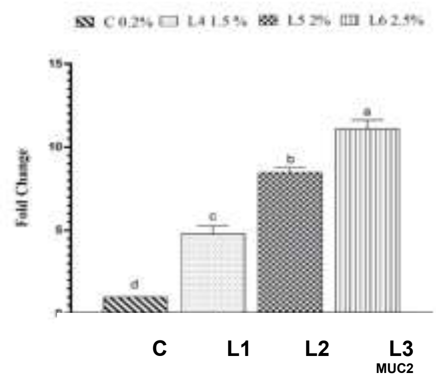


Figure (8a): Expression of MUC2 gene in small intestine of El-Salam chicken at different methionine levels.

Figure (8b): Expression Of El-Salam chicken at different lysine levels.

Treatment M1 showed the most significant (P≤0.05) increase in gene expression at (11.66±0.69) times more than control group (C) and significantly increased between other groups where averages values were (8.21±0.40) and (5.62±0.35) in both M2 and M3 respectively **(Table 11and Fig.8a &b)**. Regardless of whether lysine and methionine levels were raised, lowered, or maintained in comparison to the control group, Chai et al. (2020) found that a decrease in CP dramatically boosted MUC2 gene expression.There was no treatment where all immunity genes were up-regulated at the same time, and different amino acid treatments had varying effects on different immunity genes **(Lee et al., 2020).**

CONCLUSION:

We found that adding access amino acids methionine 0.50 or / and lysine with levels of 2.50 (%) to rations of local poultry breed AL-SALAM diet improvement growth performance (body weight rise, carcass % lysozyme activity, feed conversion rate, decreased daily feed intake and high changes of gene expressions (Atrogene-1, IGF-1, GAL6 and MUC2) compared to control group.

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