

Effect of L-Threonine supplementation on meat quality of broiler chickens

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Abstract

Supplementation of threonine above recommended value in broiler chicken diet is geared toward growth performance and meat production. However, information on its effect on meat quality which is an important parameter for sustainable poultry production is limited. Therefore, the need to evaluate the quality of the meat when threonine was fed above NRC (1994) recommendation in broiler chickens. One-day-old Ross 308 broiler chicks ($n=200$; $r=5$; 10birds/r) in a completely randomized design were allotted to four dietary treatments: Thr1=basal diet, Thr2=basal diet + NRC threonine requirement, Thr3= basal diet + 15% > NRC threonine requirement, Thr4= basal diet + 30% > NRC threonine requirement. The birds were fed ad libitum. After 6 weeks, five birds per replicate were randomly selected, euthanized, defeathered, eviscerated and cut into primal cuts according to standard procedures. Proximate composition, cooking loss, thermal shortening, sensory characteristics (9-point-hedonic scale), Thiobarbituric Acid Reactive Substances (TBARS $\mu\text{M/g}$) at 0, 7, 14 days) were accessed on the breast muscle using standard procedures. Data were analysed using ANOVA and means were separated using DUNCAN @ $p<0.05$. Moisture (66.45%) and fat (11.58%) of meat from Thr 4 were significantly higher ($P<0.05$) than 64.27%; 10.30% (Thr 1), 65.48%; 9.63% (Thr 2) and 64.04%; 10.85% (Thr3) for moisture and fat respectively. No significant differences ($p>0.05$) in cooking loss and thermal shortening. Overall acceptability (6.46) of cooked meat from Thr3 diet was similar to 5.83 (Thr2) and 6.57 (Thr 4) but significantly higher ($p<0.05$) than 5.50 (Thr1). Irrespective of storage days, TBARS (0.171 $\mu\text{M/g}$) recorded in cooked meat from Thr4 was significantly lower ($p<0.05$) than 0.201 $\mu\text{M/g}$ (Thr1), 0.201 $\mu\text{M/g}$ (Thr2) and 0.187 $\mu\text{M/g}$ (Thr3). Supplementing threonine in broiler chicken diet above the recommended value did not pose any adverse effect on the quality of the meat.

Keywords: Broiler meat quality, shelf life, broiler production, carcass quality, limiting amino acid

Running title: Dietary supplementation of Threonine on Meat Quality of Broiler Chickens



Effet d'une supplémentation en L-thréonine sur la qualité de la viande des poulets de chair

Résumé

La supplémentation en thréonine au-delà des valeurs recommandées dans l'alimentation des poulets de chair vise à améliorer les performances de croissance et la production de viande. Cependant, les informations concernant son effet sur la qualité de la viande, qui constitue un paramètre important pour une production avicole durable, sont limitées. Il était donc nécessaire d'évaluer la qualité de la viande lorsque la thréonine était administrée à des doses supérieures aux recommandations du NRC (1994) chez les poulets de chair. Des poussins de chair Ross 308 âgés d'un jour ($n = 200$; $r = 5$; 10 oiseaux/r) ont été répartis, selon un plan d'expérimentation complètement aléatoire, dans quatre traitements alimentaires : Thr1 = régime de base, Thr2 = régime de base + apport en thréonine conforme aux recommandations du NRC, Thr3 = régime de base + 15 % de thréonine en plus par rapport aux recommandations du NRC, Thr4 = régime de base + 30 % de thréonine en plus par rapport aux recommandations du NRC. Les volailles ont été nourries à volonté. Au bout de 6 semaines, cinq volailles par réplicat ont été sélectionnées au hasard, euthanasiées, plumées, éviscérées et découpées en morceaux primaires selon les procédures standard. La composition proximale, la perte à la cuisson, le rétrécissement thermique, les caractéristiques sensorielles (échelle hédonique à 9 points) et les substances réactives à l'acide thiobarbiturique (TBARS,

$\mu\text{M/g}$) à 0, 7 et 14 jours ont été évalués sur le muscle pectoral selon des procédures standard. Les données ont été analysées par ANOVA et les moyennes ont été séparées à l'aide du test de Duncan à $p < 0,05$. La teneur en eau (66,45 %) et en matières grasses (11,58 %) de la viande issue du groupe Thr 4 était significativement plus élevée ($P < 0,05$) que celle de 64,27 % ; 10,30 % (Thr 1), 65,48 % ; 9,63 % (Thr 2) et 64,04 % ; 10,85 % (Thr 3) pour la teneur en eau et en matières grasses respectivement. Aucune différence significative ($p > 0,05$) n'a été observée en termes de perte à la cuisson et de rétrécissement thermique. L'acceptabilité globale (6,46) de la viande cuite issue du régime Thr3 était similaire à celle de 5,83 (Thr2) et 6,57 (Thr4), mais significativement supérieure ($p < 0,05$) à celle de 5,50 (Thr1). Indépendamment de la durée de stockage, la valeur des TBARS (0,171 $\mu\text{M/g}$) enregistrée dans la viande cuite issue du régime Thr4 était significativement inférieure ($p < 0,05$) à celle de 0,201 $\mu\text{M/g}$ (Thr1), 0,201 $\mu\text{M/g}$ (Thr2) et 0,187 $\mu\text{M/g}$ (Thr3). L'ajout de thréonine dans l'alimentation des poulets de chair à un niveau supérieur à la valeur recommandée n'a eu aucun effet négatif sur la qualité de la viande.

Mots-clés : qualité de la viande de poulet de chair, durée de conservation, production de poulets de chair, qualité de la carcasse, acide aminé limitant

Introduction

Broiler production is a significant portion of poultry production (Maharjan et al., 2021) and nutrition is an important aspect of poultry production (Abd El-Hack et al., 2025). Efficient feed utilization and appropriate diet formulation is critical for profitable poultry production, because feed which represents the largest component of production expenditure account for approximately 60–80% of total production costs in intensive poultry production (Ajide et al., 2023; Sowunmi et al., 2022). In broiler nutrition, amino acids are essential for optimal broiler growth and protein deposition, especially in large-scale commercial operations, where precise dietary amino acid levels and appropriate ratios are necessary to fulfill bird's nutritional demands and optimize growth performance. ((Maharjan et al., 2021)). Therefore, supply of essential amino acids in broiler nutrition is of great concern and among the several amino acids, methionine, lysine and threonine are the major limiting amino acids in conventional poultry feed particularly in broiler formulations (Alabi and Adedokun 2025). Lysine plays an important role in protein synthesis, muscle development and maintenance of body tissues, thereby supporting growth and productivity (Alagawany et al., 2020) while methionine contributes to protein synthesis and

muscle accretion and also serves important metabolic functions (Alabi and Adedokun, 2025). Threonine on the other hand although is essential for protein synthesis but is particularly important for intestinal health because it is a major component of mucin, which contributes to the maintenance of the intestinal mucosal barrier and supports nutrient absorption and immune function (Bortoluzzi et al., 2020). Supplementation of threonine has also been reported to improve carcass characteristics probably because threonine is the second limiting amino acid for breast meat yield (Estalkhizir et al., 2013).

These outstanding benefits of threonine has prompted its inclusion in broiler diets above the recommended NRC (1994) value. Reports have shown that applications of threonine above NRC requirements, resulted in a better growth rate, feed utilization, carcass quality and gut health and increased ileal digestibility of amino acids and protein, and enhanced immunity (Ahmed et al. 2020). Most studies on increased inclusion of threonine in diet have only focused on its effectiveness in enhancing overall growth performance of the birds. However, one of the important considerations in sustainable poultry production is maintaining and optimizing product quality while improving production efficiency

(Yue et al., 2024) which is of utmost important to the consumers as it influences their buying decisions. Therefore, this research aimed evaluated effects of various inclusion levels of threonine in broiler diets and breast meat quality, focusing particularly on the physico-chemical and shelf-life of the meat.

Materials and methods

Site of experiment

The research was conducted at the poultry unit of the Teaching and Research Farm University of Ibadan.

Birds and management

One- day-old Ross 308 unsexed broiler chicks (n=200) were procured from a reliable hatchery in Ibadan, Oyo State. The chicks were weighed and wing banded on arrival, provided with anti-stress and multivitamins, and then distributed randomly (n=200; r=5; 10 birds/r). The feed was formulated based on the NRC recommendation (Table 1) and were fed to the birds unrestricted alongside with clean water for a period of 6weeks. were supplied unrestricted with water to the birds. All vaccination schedule and important housing management practices were strictly followed.

Table 1: Ingredients composition (g/100gDM) of experimental diets

Ingredients	Pre-starter	Starter	Finisher
Maize	51.64	57.00	60.10
Soyabean meal	40.00	35.00	31.00
Fish meal	2.10	1.52	1.13
Soya oil	2.38	2.60	3.90
Methionine	0.18	0.18	0.17
Lysine	0.10	0.10	0.10
Limestone	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00
Table salt	0.25	0.25	0.25
Starter premix	0.25	0.25	0.25
Toxin binder	0.10	0.10	0.10
TOTAL	100.00	100.00	100.00
<i>Calculated nutrient</i>			
Crude protein (%)	23.39	21.17	19.35
ME (kcal./kg)	2963.98	3016.18	3115.00
Fat (%)	5.23	5.36	6.51
Fibre (%)	3.98	3.79	3.60
Calcium (%)	1.09	1.06	1.03
Available phosphorus (%)	0.34	0.34	0.32
Methionine (%)	0.54	0.51	0.47
Lysine (%)	1.38	1.23	1.11
Threonine (%)	0.87	0.79	0.73

Experimental design

The experiment was completely randomized with 4 dietary treatments and each treatment replicated 5 times with ten birds per replicate. The diet layout is displayed below and the amount (g) of L-threonine included in the diet is shown on Table (2)

0 % NRC: Basal diet + 0% threonine

NRC: Basal diet + NRC threonine recommendation

15% > NRC: Basal diet + 15% > NRC threonine recommendation

30% > NRC: Basal diet + 30% > NRC threonine recommendation

Table 2: Inclusion levels of threonine (g/kg) used in the study

Dietary treatments	L-threonine inclusion levels (g)		
	Pre-starter	Starter	Finisher
Basal diet only	0	0	0
Basal diet +threonine (NRC)	0.80	0.77	0.74
Basal diet +threonine 15% > NRC	0.92	0.89	0.85
Basal diet +threonine 30% > NRC	1.04	1.00	0.96

Carcass preparation

On day 42 of the feeding trials, three birds from each replicate were selected randomly. The birds

were euthanized, defeathered (hot defeathering) and eviscerated following standard procedures. Each carcass (each treatment separately) was cut into primal cuts and individually weighed and recorded. The breast muscle was then removed and put into separate labelled ziploc bags for further analysis.

Breast meat quality assessment

Proximate composition

This was carried out according to the AOAC (2019) procedural method. The breast meat nutrient composition such as moisture, crude protein, fat and ash contents were determined.

Cooking loss and thermal shortening percentages

For cooking loss meat samples were trimmed into a uniform meat sample (50-100 g broiler breast) and weighed. The weighed sample was cooked until the internal temperature reaches 70-75°C (20-30 min). After cooking, the samples were re-weighed and the percentages loss due to cooking was calculated. Thermal shortening measurement involved cutting the meat samples to a uniform meat strip (5 cm × 2 cm × 1 cm). The samples were subsequently immersed in pre-heated water and subjected to heat at 20 minutes cooking period. After cooling, the cook length was measured using a ruler and the change in length was calculated.

Cooking loss (%)

$$= \frac{\text{Initial weight of meat} - \text{final weight of meat}}{\text{Initial weight of meat}} \times 100$$

Thermal shortening (%)

$$= \frac{\text{Length of raw meat} - \text{length of cooked meat}}{\text{Length of raw meat}} \times 100$$

Panelist sensory assessment

The assessment of the sensory characteristics of the cooked breast meat was carried out as described by Lukman *et al.* (2009). Twenty individuals made up of semi-trained students of the Department of Animal science, University of Ibadan, Oyo State, Nigeria. were used to investigate the sensory characteristic of the cooked meat. The sensory parameters assessed

were flavour, tenderness, juiciness, texture, colour on a 9-point hedonic scale (9 = like extremely and 1 = dislike extremely). Water and biscuits were offered to panelists to bite, chew and rinse their palate in order neutralise any lingering flavours and overlap of treatments.

Texture Profile Analysis

Meat samples were cut into 10mm cubes from the central region. Texture profile analysis (TPA) was performed using a texture analyzer (TA-XT. plus, Stable Micro Systems Ltd., UK) with a cylindrical probe following the method described by (Yuliarti *et al.*, 2021). The parameters measured were hardness, chewiness, cohesiveness, resilience, adhesiveness and springiness.

Thiobarbituric Acid Reactive Substances (TBARS) evaluation

The degree of lipid oxidation during refrigerated storage of the breast meat fillet was assessed through the Thiobarbituric Acid Reactive substances (TBARS) assay technique on 0, 7 and 14 days of storage following the procedures described by Azman *et al.* (2014). Meat samples were placed in a beaker and 50 ml of a chilled (4°C) solution containing 20% trichloroacetic acid in 1.6% phosphoric acid was added to it and the solution was mixed thoroughly for two minutes. Another 50 ml of chilled distilled water (4°C) was then added to the solution and the mixture blended for 30 seconds. The resulting slurry was filtered through Whatman No. 1 filter paper into a 100 ml cylinder. Five mL of the filtrate was transferred to a test tube, and an equal volume of freshly prepared 0.02 M 2-thiobarbituric acid solution was added. The test tubes were kept in the dark at room temperature (25°C) for 15 hours to allow the colour reaction to develop. The colour intensity was measured using a spectrophotometer at 532 nm to determine the TBARS values which is expressed as milligrams of malondialdehyde (MDA) per kilogram of meat (mgMDA/kg).

Total Volatile Nitrogen Base (TVB-N) determination

A 100g of fresh raw meat sample was treated with 300ml of 5% Trichloroacetic acid solution, the mixture was centrifuged, and then a 5mL aliquot of the clear extract was subjected to steam distillation in a Markham apparatus with 5mL of 2M NaOH solution. The distillate was collected in 0.01M HCl solution with rosolic indicator and titrated with 0.01M NaOH until a faint pink colour appeared, indicating the end point. The amount of the total volatile basic nitrogen in the raw meat was calculated using the following equation and the result expressed as milligrams of nitrogen per 100 grams of sample (mgN/100g).

$$TVBN \text{ mgN/100g} = (M)(VB - VS)(14)(300 + W/5)$$

Where:

VB = Milliliters of NAOH used for blank titration

W=water content of samples in grams per 100grams

M = molarity of NAOH standard solution

VS = Milliliter of NAOH used for sample titration

Statistical analysis

Data were analysed using One Way ANOVA. The differences between means were separated using New Duncan's Multiple Range test at a significance level of $p \leq 0.05$.

Results and discussion

There were no significant differences in most of the carcass traits measured (Table 3) except for the head and neck (Table 3). Head (3.37%) of broiler meat from 0% NRC diet is similar to 2.83% (NRC) but significantly higher ($P < 0.05$) than 2.45 (15% >NRC) and 2.33 (30% >NRC). Neck (5.12%) of broiler meat from 30% > NRC diet is similar ($P > 0.05$) to 4.25 % (NRC) and 4.80 (15% > NRC) significantly higher ($P < 0.05$) than 3.57 % (0% NRC).

Table 3: Relative weight (%) of some carcass traits of broiler chicken fed L- threonine at varying levels of inclusion

Parameters (%)	0%NRC	NRC	15%>NRC	30%>NRC	SEM	P-value
Head	3.37 ^a	2.83 ^{ab}	2.45 ^b	2.33 ^b	0.13	0.01
Neck	3.57 ^b	4.25 ^{ab}	4.80 ^{ab}	5.12 ^a	0.20	0.02
Breast	20.84	21.06	20.10	19.97	0.53	0.88
Drumstick	10.32	10.14	10.00	10.20	0.14	0.90
Shank	4.50	4.28	4.32	4.63	0.11	0.68
Wings	8.79	8.65	8.36	8.37	0.18	0.81
Back	16.59	14.59	15.80	14.51	0.32	0.04
Thigh	9.46	9.47	10.97	10.85	0.33	0.18

^{a,b}: Means on the same row with different superscripts are significantly ($P < 0.05$) different

0%NRC= Basal diet + No Threonine

NRC= Basal diet + Threonine NRC recommendation

15%NRC= Basal diet + Threonine 15%>NRC recommendation

30%NRC= Basal diet + Threonine 30% > NRC recommendation

The age, strain and sex of broiler, crude protein content of feed, type and proportion of dietary ingredients used are some major factors that determine the threonine requirement for carcass

yield in broiler birds (Barkley and Wallis, 2001). It would have been expected that there will be improvement in the carcass yields (particularly the primal cuts) due to the increase amount of the essential amino acids (threonine) in the diet as opined by Estalkhizir et al. (2013). However, this study elucidates that yields from the primal cuts (apart from the neck cut) were not positively affected irrespective of the level of threonine supplementation. This insignificant change might be attributed to the fact that other variables

particularly the strain and sex which also determine the threonine requirement for carcass yields in broiler birds (Barkley and Wallis, 2001) are not considered in this study. These findings slightly agreed with Rezaeipour *et al.* (2012), who observed no significant impact on carcass traits of broilers during starter phase when threonine inclusion in broiler's diet was increased. The outcome of the present study agreed with Rezaeipour *et al.* (2014) that incorporating up to 544g threonine per ton of diet had no significant impact on the growth performance or carcass characteristics of commercial broiler chickens and, Upadhya *et al.* (2021) who observed no significant increase in the carcass traits of pig fed higher threonine

levels in their diets. The results of this study contradicted the observations of Kheiri and Alibeyghi (2017) on increased carcass weights and higher breast and thigh yields from chickens fed 120% threonine above NRC recommendation while El-Faham *et al.* (2017) reported a 2.8% and 1.6% increase in breast and drumstick weights, respectively when dietary threonine level of broiler birds was increased by 0.87% and Al-Hayani (2017) who reported an enhanced carcass and breast weights of 3.7% and 2.3%.

No statistical differences ($P>0.05$) in cooking loss (25.13-28.94%) and thermal shortening (26.74-34.06%) of the cooked meat (Table 4) were observed.

Table 4: Cooking loss and thermal shortening of meat from broiler chicken fed varying inclusion of L- threonine diet

Parameters (%)	Inclusion levels (L-threonine g/kg)				SEM	P-value
	0% NRC	NRC	15% >NRC	30% >NRC		
Thermal shortening	29.89	31.89	34.06	26.74	3.81	0.5422
Cooking loss	25.13	28.94	27.83	25.28	1.86	0.4053

Means with same superscript on the same row are significantly not different ($P>0.05$)

0%NRC= Basal diet + No Threonine

NRC= Basal diet + Threonine NRC recommendation

15%>NRC= Basal diet + Threonine 15%>NRC recommendation

30%>NRC= Basal diet + Threonine 30% > NRC recommendation

Although, nutritional manipulation is a viable approach used by producer to improve the growth performance of animals, however, the quality of the resulting meat is also of utmost importance to the meat industry, thus the need to evaluate the quality of the meat produced. The present study showed that increasing the level of threonine in the broiler diet did not have any significant effect on the thermal shortening and cooking losses of the meat after heat treatment. This finding followed the trend of cooking losses reported by Lilly *et al.* (2011) that no significant differences was observed in the cooking loss of meat from broiler chicken fed varying amino acid density.

The higher cooking loss observed in this study when compared with 21.5-22.3% reported by Lilly *et al.* (2011) could be due to differences in the cooking method because Lilly *et al.* (2011) used oven cooking method which might have resulted in the lower cooking loss when compared to boiling method that was used in this study. Furthermore, Belal *et al.* (2008) also reported no significant cooking loss in the meat of Awassi Ram fed varying level of methionine (sulphur containing amino acid) in their finishing diet.

The moisture (66.45%) and fat (11.58%) contents (Table 5) of broiler meat from 30%>NRC were significantly higher ($P<0.05$) while protein (21.36%) of meat from broiler fed 15%> NRC was significantly higher ($P<0.05$). The ash (1.96%) of meat from broiler fed 15%> NRC was similar ($P>0.05$) to 1.86 (NRC) but lower ($P<0.05$) than 1.76% (0%NRC) and 1.72 (30%>NRC).

Table 5: Proximate composition of raw meat from broiler chicken varying inclusion levels of L-threonine in the diet

Parameters (%)	Inclusion level (L-threonine g/kg)				SEM	P-value
	0% NRC	NRC	15%>NRC	30%>NRC		
Moisture	64.27 ^c	65.48 ^b	64.04 ^d	66.45 ^a	0.05	<0.0001
Protein	20.14 ^b	19.24 ^c	21.36 ^a	17.72 ^d	0.06	<0.0001
Fat	10.30 ^c	9.63 ^d	10.85 ^b	11.58 ^a	0.08	<0.0001
Ash	1.76 ^b	1.86 ^{ab}	1.96 ^a	1.72 ^b	0.05	0.0350

^{a,b,c,d}: Means on the same row with different superscripts are significantly (P<0.05) different

The inclusion of threonine above NRC recommendation in broiler chicken diet had a notable effect on the proximate composition of the resulting broiler meat. The inclusion of threonine above 30% NRC recommendation led to an increase in moisture and fat contents but decreased ash and surprisingly lower protein contents of the meat. It was assumed that meat from broiler with higher threonine inclusion in the diet would have an increased protein content because of the assumption of deposition of higher protein in their tissue (Ospina-Rojas *et al.*, 2013). However, the reversed was observed where increasing threonine above 30% NRC (1994) recommendation resulted in a reduced protein content of the broiler meat. The result of this study contradicts the findings of Corzo *et al.* (2005) on reduction in meat fat as amino acid

density of feed increases and Pokko-Aikins *et al.* (2021) who also did not observe any notable effect in the proximate composition of broiler meat when broiler chicken was fed varying level of methionine.

There was no significant differences (P>0.05) in colour (Table 6) while aroma (4.43; 4.53) and juiciness (6.20; 6.63) of cooked broiler meat from broiler fed 15% and 30% > NRC (1994) respectively were similar and significantly higher (P<0.05). Taste (6.50) of 15%>NRC cooked meat is similar to 6.43 (30%>NRC) and significantly higher (P<0.05) while overall acceptability of 30%>NRC (6.57) cooked meat is similar (P>0.05) to 6.46 (15%>NRC) but significantly higher (P<0.05) than 5.83 (NRC) and 5.50 (0%NRC).

Table 6: Panelists sensory assessment of cooked meat from broiler chicken fed graded levels of L-threonine diets

Parameters	Inclusion level (L-threonine g/kg)				SEM	P-value
	0%NRC	NRC	15%>NRC	30%>NRC		
Colour	6.70	6.17	6.43	6.60	1.16	0.6160
Aroma	3.20 ^b	3.33 ^b	4.43 ^a	4.53 ^a	1.42	0.0143
Flavour	3.67 ^b	4.00 ^b	5.20 ^a	4.63 ^{ab}	1.32	0.0009
Taste	5.77 ^b	5.87 ^b	6.50 ^a	6.43 ^{ab}	0.92	0.0533
Juiciness	4.77 ^b	4.83 ^b	6.20 ^a	6.63 ^a	1.24	0.0001
Overall acceptability	5.50 ^c	5.83 ^{bc}	6.46 ^{ab}	6.57 ^a	0.91	0.0036

^{a,b,c}: Means on the same row with different superscripts are significantly (P<0.05) different

0%NRC= Basal diet + No Threonine

15%NRC= Basal diet + Threonine 15%>NRC

NRC= Basal diet + Threonine NRC

recommendation

recommendation

30%NRC= Basal diet + Threonine30% > NRC recommendation

Dietary feeding of threonine above NRC recommendation in this study did not introduce any abnormal visual colour to the cooked meat. However, increasing threonine above NRC (1994) recommendation had significant influence on flavour, juiciness, aroma, and overall acceptability of the cooked meat. The aroma and juiciness of meat from broiler chickens fed threonine at 30% above NRC (1994) recommendation was higher when compared with others. This might be attributed to the higher moisture and fat contents of this meat. This is because water in meat and meat products are important component that has significant impact on various parameters such as organoleptic, structural-mechanical properties of raw

materials, quality and storability of the ready-to-consume food products (Kudryashov and Kudryashova, 2023). For instance, water retained in and the amount of fat contained in cooked meat are factors that contributes to meat juiciness (Malgwi *et al.*, 2022; Ji *et al.*, 2024). This might imply that increasing the threonine content in broiler diet up to the inclusion level reported in this study might aid water retention in the cooked meat.

No significant differences ($P>0.05$) in the hardness and cohesiveness (Table 7) of the cooked meat irrespective of the diets. Adhesiveness (0.52g/sec) and resilience (58.06%) of cooked meat from birds fed NRC (1994) were significantly higher ($P<0.05$). Springiness (91.37%) of meat from 30%>NRC and chewiness (6838.60g) of meat from 0%NRC were significantly higher ($P<0.05$).

Table 7: Textural properties of raw meat from broiler chicken fed L-threonine diets

Parameters	Inclusion level (L-threonine g/kg)				SEM	P-value
	0%NRC	NRC	15%>NRC	30%>NRC		
Hardness (g)	13.49	14.27	13.03	13.80	1.45	0.9397
Adhesiveness(g/sec)	0.20 ^{ab}	0.52 ^a	0.36 ^{ab}	0.07 ^b	0.21	<0.001
Resilience (%)	36.05 ^b	58.06 ^a	34.65 ^b	37.30 ^b	0.93	<0.001
Cohesiveness	0.74	0.94	0.75	0.76	0.09	0.3497
Springiness (%)	85.96 ^{ab}	73.07 ^c	81.86 ^b	91.37 ^a	1.90	0.0009
Chewiness (g)	6838.60 ^a	4427.00 ^b	4833.10 ^b	4614.90 ^b	193.90	<0.0001

^{a,b}: Means on the same row with different superscripts are significantly ($P<0.05$) different

0%NRC= Basal diet + No Threonine

NRC= Basal diet + Threonine NRC recommendation

15%NRC= Basal diet + Threonine 15%>NRC recommendation

30%NRC= Basal diet + Threonine30% > NRC recommendation

Texture profile analysis is a double compression test which mimic the action of chewing that usually occur in the mouth during mastication (Khule *et al.*, 2024). It is usually combined with subjective (human) sensory perception of food to obtain more comprehensive information on the texture of the food (Funami and Nakauma, 2022).

It measures different properties of the food such as hardness which is the force required to deform a food, cohesiveness which measures the internal bond strength (how a food stays together), chewiness which refer to the energy required to chew and break down the food to a swallowing state/ consistency (Tian *et al.*, 2024) etc. No noticeable difference was observed in the cohesiveness and hardness of the cooked meat. However, there were differences in the chewiness of the meat as meat from broiler chickens fed higher threonine level had lower chewiness. This implies that less effort/energy is required to turn these meats into a swallowing state. This could be

as a result of the high moisture content and or probably the high fat content contained in these meats. The springiness of the cooked meat did not follow a particular pattern, but it was observed that meat from broiler chickens fed 30% above the NRC recommendation have the ability to quickly regain back its original shape after

compression as indicated with the high springiness value.

Irrespective of the days, the TBARS 0.171 µM/g recorded in cooked meat of broiler fed threonine 30% > NRC was significantly lower ($P < 0.05$) than 0.202 µM/g (0%NRC), 0.201 µM/g (15%>NRC) and 0.187 µM/g (NRC).

Table 8: Effect of treatments, storage and their interaction on Thiobarbituric acid reactive substances produced during storage of raw meat from broiler chicken fed varying levels of L-threonine diets

Week	Inclusion level (L-threonine g/kg)	TBARS (µM/g) Estimate
	0%NRC	0.202 ^a
	15%>NRC	0.201 ^a
	NRC	0.187 ^b
	30%>NRC	0.171 ^c
	SEM	0.001
	P-value	<0.0001
0		0.06 ^c
1		0.17 ^b
2		0.34 ^a
	SEM	0.001
	P-value	<0.0001
2	NRC	0.385 ^a
2	0%>NRC	0.368 ^b
2	15%>NRC	0.344 ^c
2	30%>NRC	0.255 ^d
1	15%>NRC	0.184 ^e
1	30%>NRC	0.183 ^e
1	NRC	0.163 ^f
1	0%>NRC	0.145 ^g
0	0%>NRC	0.092 ^h
0	30%>NRC	0.0743 ⁱ
0	NRC	0.054 ^j
0	15%>NRC	0.034 ^k
	SEM	0.001
	P-value	<0.0001

^{a,b,c,d,e,f,g,h,i,j,k} : Means in the same rows with different superscripts are statistically different ($P < 0.05$)

0%NRC= Basal diet + No Threonine

NRC= Basal diet + Threonine NRC recommendation

15%NRC= Basal diet + Threonine 15%>NRC recommendation

30%NRC= Basal diet + Threonine 30% > NRC recommendation

The quality of meat directly influences consumer acceptability and it is also crucial for the economic success of the poultry industry

(Song *et al.*, 2025) therefore, the need to assess the keeping quality of meat. The quality of food during storage especially for lipid containing food such as meat can be ascertained through the extent of lipid oxidation that occurred in it which is usually measured by the amount of Thiobarbituric Acid Reactive Substances (TBARS).

The TBARS comprises of compounds such as malondialdehyde (MDA) and other related aldehydes, generated during lipid peroxidation of fatty food (Domínguez *et al.*, 2019). The result from this study showed that although the levels of TBARS in the meat during storage did not show a specific direction. However, at the end of the frozen storage days, meat from broiler chickens fed threonine at 30% above NRC recommendation have a lower TBARS value when compared to other meats implying reduced lipid oxidation. This might probably mean that at this inclusion level, the antioxidant status of the broiler chicken improved which is now reflected

in the muscle tissue. This result agrees with the observation of Chen *et al.* (2017) who reported an increase in oxidative status of broiler chicks during the starter phase when fed with dietary threonine above NRC recommendation. Again, it would have been expected that meat from broiler fed threonine 15% above NRC (1994) recommendation will also have lower TBARS because of the low crude fat it contained because the fat content of a food is one of the factors that influences lipid oxidation (Domínguez *et al.*, 2019). This result is in line with the research carried out by Ospina-Rojas *et al.* (2018), who observed a linear relationship between meat lipid oxidation and standardized ileal digestible threonine supplementation in broiler chicken.

Irrespective of the storage day, the TVBN 10.11mgN/100g recorded in cooked meat from broiler fed 30%>NRC was significantly lower ($P<0.05$) than 10.67mgN/100g (15%>NRC), 11.04 mgN/100g (NRC) and 10.25 mgN/100g (0% NRC).

Table 9: Effect of treatment, storage and their interaction on Total volatile Basic Nitrogen (TVB-N) accumulated during storage of raw meat from broiler chicken fed varying level of supplemented L-threonine diets

Week	Treatment	TVB-N Estimates (mgN/100g)
	0% NRC	10.25 ^c
	NRC	11.04 ^a
	15%>NRC	10.67 ^b
	30%>NRC	10.11 ^d
	SEM	0.19
	P-value	<0.0001
0		7.19 ^c
1		9.52 ^b
2		14.84 ^a
	SEM	0.19
	P-value	<0.0001
2	15%>NRC	15.09 ^a
2	NRC	14.89 ^{ab}
2	0%>NRC	14.75 ^b
2	30%>NRC	14.62 ^b
1	NRC	9.87 ^c
1	0%>NRC	9.67 ^{cd}
1	30%>NRC	9.53 ^d
1	15%>NRC	9.02 ^e
0	NRC	8.37 ^f
0	15%>NRC	7.90 ^g
0	0%>NRC	6.34 ^h
0	30%>NRC	6.19 ^h
	SEM	0.19
	P-value	<0.0001

^{a,b,c,d,e,f,g,h,i,j,k}. Means in the same rows with same superscripts are statistically not different (P<0.05)

0% NRC= Basal diet + No Threonine

NRC= Basal diet + Threonine NRC recommendation

15%NRC= Basal diet + Threonine 15%>NRC recommendation

30%NRC= Basal diet + Threonine 30% > NRC recommendation

Total Volatile Basic Nitrogen is the total amount of primary, secondary, and tertiary amines, including volatile amines and toxic nitrogen compounds contained in a food (Li *et al.*, 2015). It is commonly used as an objective biomarker to assess meat integrity, safety, and edibility (Ma *et*

al., 2013). An increase in TBV-N level indicates the degree of protein breakdown due to microbial proliferation and proteolytic enzyme activity (Hassan and Omama, 2011). It would have been expected that the TVB-N contained in meat from broiler fed 30% threonine above NRC (1994) recommendation would be higher. This is because threonine is an important constituent in protein synthesis and maintenance of body protein turn over (Ton *et al.*, 2013) and it is assumed that increased threonine might result in increased protein accumulation and consequently its breakdown during storage. However, it was observed that irrespective of the storage duration,

broilers fed 30% threonine above NRC (1994) had a relatively suppressed TVB-N value suggesting reduced protein degradation thus enhanced meat freshness. The lower TVB-N recorded could be as a result of the lower crude protein (proximate composition) recorded in this meat. This implies that lower protein is available in the meat for degradation thus enhance freshness of meat. Again, TVB-N accumulation in muscle is not only limited to available protein but also the activity of microorganisms in the meat. The findings of this research affirmed the result of Diaa *et al.* (2021) which illustrates that supplementing higher sulphur amino acid in form of methionine in broiler diet resulted to lower TVB-N accumulation.

Conclusion

The results from this study showed that although the National Research Council's recommended threonine levels are sufficient for broiler growth. The improved proximate composition and panelist acceptability of cooked meat coupled with the low thiobarbituric acid reactive substances level in the meat during refrigerated storage showed that supplementing threonine beyond the recommended levels in broiler diet do not have adverse effect on the meat quality.

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