Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2023.043

RESEARCH ARTICLE

# Effects of Co-Supplementation of β-Galacto-Oligosaccharides and Methionine on Breast Meat Quality, Meat Oxidative Stability and Selected Meat Quality Genes in Broilers

Sohrab Ahmad[1,4], Muhammad Shahbaz Yousaf[1*], Sajid Khan Tahir[1], Muhammad Afzal Rashid[2], Khalid Abdul Majeed[1], Mahrukh Naseem[3], Mohsin Raza[4], Zafar Hayat[5], Abia Khalid[1], Hafsa Zaneb[6] and Habib Rehman[1*]

[1]Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Pakistan; [2]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan; [3]Department of Zoology, University of Balochistan, Quetta, Balochistan, Pakistan; [4]Department of Basic Sciences, Khan Bahadar Choudhry Mushtaq Ahmed College of Veterinary and Animal Sciences, Narowal, Pakistan; [5]Department of Animal Sciences, College of Agriculture, University of Sargodha, Sargodha Pakistan; [6]Department of Anatomy and Histology, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding author: habibrehman@uvas.edu.pk; drmshahbaz@uvas.edu.pk

ARTICLE HISTORY (23-049)

| | |
|---|---|
| Received: | February 12, 2023 |
| Revised: | May 01, 2023 |
| Accepted: | May 22, 2023 |
| Published online: | June 18, 2023 |

**Key words:**
Amino acid
Broilers
Meat quality
Methionine
Prebiotic
Supplementation

**A B S T R A C T**

The present study was designed to investigate the effects of co-supplementation of β-galacto-oligosaccharides (β-GOS), a prebiotic, and methionine on selected meat quality traits, and the pathways governing breast muscle formation and degradation in broilers. Day-old broilers (n = 288) were distributed in a 3×2 factorial design (6 groups and 6 replicates/group) based on three dietary levels of β-GOS (0, 0.2 and 0.5%) and two levels of methionine (0.5 and 1.0%). On day 35, two birds per replicate (12 birds per group) were exsanguinated to collect breast muscle samples for analysis. Results showed that β-GOS supplementation reduced the initial $pH_{15min}$ ($P<0.05$), rate of pH change, cooking loss ($P<0.05$), and muscle fiber diameter ($P<0.05$) with a concomitant increase in the muscle catalase level ($P<0.05$), and fiber density ($P<0.05$). Methionine (1.0%) downregulated the expression of MAFbx ($P<0.05$) and MuRF1 ($P<0.05$) genes. Methionine (0.5%) supplementation significantly decreased the drip loss and upregulated the expression of MyoD ($P<0.05$) and M-CK ($P<0.05$) genes. We conclude that though β-GOS and methionine co-supplementation did not show any promising effects on meat quality in broilers, still, both β-GOS and methionine partially improved the selected breast meat traits (decreased $pH_{15min}$, ΔpH, elevated antioxidant activity, lower cooking loss, and higher muscle fiber density) and breast muscle degradative changes (downregulating the expressions of MuRF1 and MAFbx genes) respectively.

**To Cite This Article:** Ahmad S, Yousaf MS, Tahir SK, Rashid MA, Majeed KA, Naseem M, Raza M, Hayat Z, Khalid A, Zaneb H and Rehman H, 2023. Effects of co-supplementation of β-galacto-oligosaccharides and methionine on breast meat quality, meat oxidative stability, and selected meat quality genes in broilers. Pak Vet J, 43(3): 428-434. http://dx.doi.org/10.29261/pakvetj/2023.043

## INTRODUCTION

Broiler meat is of high nutritive value due to the presence of several bioactive compounds (conjugated linoleic acid, vitamins, antioxidants, and polyunsaturated fatty acids) that have beneficial effects on the human body (Petracci and Cavani, 2012). After meeting the higher quantitative demands of chicken meat, scientific studies nowadays focus on improving broiler meat quality.

Prebiotics are among the non-antibiotic growth promoters introduced after the European Commission banned the use of antibiotic growth promoters in 2006. Prebiotics are indigestible oligosaccharides that enter the birds' hindgut and are fermented by the gut microbiota into short-chain fatty acids (Rehman *et al.,* 2007). β-galactooligosaccharides (β-GOS), produced by the action of Lactobacillus-derived galactosidases, contain β (1-6) and β (1-3) glycosidic bonds (Ashraf *et al.,* 2017). Our previous studies demonstrated that β-GOS supplementation improved the production performance, villus height, villus surface area, and caecal lactobacillus count in broilers (Yousaf *et al.,* 2016; Ashraf *et al.,* 2017; Yousaf *et al.,* 2017). In another study, GOS supplementation has been found to increase the breast

muscle weight and fiber diameter in broilers and had a higher nutritional index (n-6/n-3, PUFA/SFA), which is beneficial to human health (Tavaniello *et al.,* 2020). Recently, GOS has been acknowledged for its protective role against oxidative stress (Tian *et al.,* 2022).

Methionine, an essential amino acid, has a stimulatory effect on various growth factors, including insulin-like growth factor (IGF-1) and growth hormone, which are key factors in protein synthesis (Vesco *et al.,* 2013). Methionine deficiency also negatively affects meat quality traits and the oxidative status of breast meat in broilers (Wen *et al.,* 2017). Various studies revealed that dietary methionine supplementation improved muscle functionality, cooking yield, oxidative stability, and higher breast fillet percentage in broilers (Poko-Akins *et al.,* 2022). The addition of higher levels of methionine in the broilers' paternal diet has been found to decrease the pH and improved the tenderness, drip loss, shear force and meat color of offspring (Elsharkawy *et al.,* 2021). Furthermore, methionine administration inhibits the expression of muscle atrophy-related genes (ubiquitin ligases) including MAFbx, and tends to promote muscle deposition by upregulating the myogenic differentiator (MyoD) and myogenin mRNA expression (Wu *et al.,* 2019).

From the aforementioned discussion, it appears that dietary inclusion of either β-GOS or methionine potentially influences the growth performance and meat quality of broilers. However, to the best of our knowledge, information regarding the dietary inclusion of both β-GOS and methionine as growth promoters in broilers is not available. Therefore, we presumed that combined supplementation of β-GOS and methionine in the broiler's diet would improve the musculoskeletal growth and intestinal health of broilers than individual inclusion. To test our assumption, we conducted a study to explore the potential role of co-supplementation of β-GOS and methionine in various doses in broilers and found that co-supplementation had positive effects on improving production performances and better intestinal health in terms of deeper jejunal crypts (Ahmad *et al.,* 2022). In continuation of our previous study, currently, we are reporting the influence of the co-supplementation of β-GOS and methionine on selected parameters of meat quality (pH, water-holding capacity, cooking loss, and the meat microarchitecture), the redox status of the meat, and the expressions of meat quality genes (MAFbx, MuRF1, MyoD, and M-CK) in broilers.

## MATERIALS AND METHODS

This study was approved by the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore-Pakistan (DR/324/02/04/2018).

**Experimental diet, design, and husbandry:** This research work is the continuation of our previously published study, Ahmad *et al.* (2022), and has been carried out on the samples collected from the same birds. The experimental design, management, and diets had already been described earlier (Ahmad *et al.,* 2022). Briefly, day-old, broiler chicks (n = 288) were arranged in a 3×2 factorial arrangement. Dietary treatments included three levels of β-GOS (0, 0.2 and 0.5%) and two levels of methionine (0.5 and 1.0%). The birds were randomly distributed into six experimental groups, each group with six replicates. Each replicate contained eight birds (n = 8), thus each group had forty-eight birds (n = 48 per group). On day 35, two birds from each replicate (12 birds/group) were randomly selected and exsanguinated for obtaining breast muscle samples.

**Physical characteristics of the meat:** Muscle pH was determined at two points, firstly after finishing the slaughtering ($pH_{15min}$), and secondly 24 hours ($pH_{24hrs}$) after slaughter. The rate of change in the pH, ΔpH (ΔpH = $pH_{15min}$ - $pH_{24hrs}$) was calculated according to Ma *et al.* (2021). For muscle pH, the penetrating glass electrode of a portable pH meter (PCE-228 M pH meter, Southampton, UK) was inserted at least 1 cm into the breast muscle and the reading was recorded once stable. Percent drip loss was estimated using the gravimetric method described by Honikel (1998). The cooking loss was calculated 24 hours after slaughter as described earlier (Cheng *et al.,* 2018).

**Muscle redox status:** A 100 mg breast muscle sample was homogenized in 1.0 mL ice-cold phosphate buffer and then, centrifuged at 3000 × g for 20 minutes. From the resultant supernatant muscle malondialdehyde (MDA) and catalase levels were estimated according to the methods described by Ohkawa (1979) and earlier Hadwan *et al.* (2016) respectively.

**Muscle histology:** For muscle, histomorphometry pictures were taken from the Hematoxylin and Eosin (H&E) tissue sections with a camera-fitted microscope (Labomed Inc., California, USA) and morphometry software (Prog Res®2.7.7 Capture Prog Camera Control Software, Jenoptik Optical Systems, Jena, Germany). Muscle fiber density was determined in a circle of 0.5mm radius drawn on the pictures taken at 4X. The circle was split into half and fibers present inside the right half were counted. Muscle fiber diameter was estimated from the slide pictures taken at 10X. A rectangle of equal dimensions was drawn on each image and was then split into two rows and five columns (10 boxes). The fibers present in the alternate boxes were chosen and the averages of their vertical and horizontal diameters were reported as muscle fiber diameter. For muscle fascicle diameter estimation, three complete fascicles were selected in pictures taken at 4X, and their vertical and horizontal measurements were used (Khan *et al.,* 2022).

**mRNA extraction and quantification of meat quality genes using RT-PCR:** In this procedure, mRNA extracted from the breast muscle using Trizol® reagent (Invitrogen, Karlsruhe, Germany) was reverse transcribed for complementary DNA (cDNA) synthesis using the Revert-Aid first-strand cDNA synthesis kit (Thermo Scientific™, Waltham, USA). The cDNA along with the oligonucleotide primers was then subjected to RT-PCR using SYBER green maxima PCR kit (Thermo Scientific™, USA). The primer details for the target genes (MAFbx, MuRF1, MyoD, and M-CK) and housekeeping gene (GAPDH) and reaction protocol were as per Li *et al*. (2011) (Table 1). The relative expression was determined using the $2^{-\Delta\Delta CT}$ method, as described by Livak and Schmittgen (2001).

**Statistical analysis:** Data were analyzed using the factorial ANOVA under the GLM procedure in SPSS (Version 20.0, IBM Inc. USA), and presented as Means ± SE. Tukey's post hoc test was applied to measure the group differences at P<0.05. The correlations between the meat pH and meat quality genes were determined using Pearson's correlation.

## RESULTS

The effects of co-supplementation on various meat quality parameters are shown in Table 2. The β-GOS had significant main effects on muscle pH determined after slaughtering ($pH_{15min}$) and ΔpH as shown in Table 2. Both $pH_{15min}$ and ΔpH decreased (P<0.01) in the broilers fed diets supplemented with 0.2% and 0.5 % β-GOS. The cooking loss also decreased (P<0.05) in the β-GOS-supplemented broilers. Methionine supplementation showed a significant main (P<0.01) effect on the drip loss percentage, which was lower (P<0.05) in the broilers fed 0.5% methionine in comparison to those fed 1.0% methionine (Table 2). The breast meat $pH_{24hrs}$ remained the same in all the treatment groups. In addition, β-GOS and methionine were shown to have no interaction effects on breast meat pH, drip loss, and cooking loss.

Similarly, β-GOS (0.2 and 0.5%) supplementation increased (P< 0.05) muscle fiber density compared to the broilers fed 0% β-GOS. On the other hand, the muscle fiber diameter decreased in the broilers fed β-GOS added diets in contrast to the non-β-GOS added diet. The treatments did not affect muscle fascicle diameter. Furthermore, methionine supplementation had no effects on the histomorphometric properties of breast muscles (Table 2, Fig. 1).

As far as the breast muscle redox status is concerned, the catalase activity was higher (P<0.01) in the β-GOS 0.5% fed birds compared to the 0% and 0.2% β-GOS fed birds. The muscle MDA content remained unaffected. Similarly, methionine supplementation did not influence the redox status of breast muscle (Table 3).

Results related to gene expression following the supplementations are shown in Fig. 2. Methionine (1.0%) supplementation reduced the expression of MAFbx (P<0.01) and MuRF1 (P<0.05) in broilers. In contrast, feeding 0.5% methionine increased the expression of MyoD (P<0.05) and M-CK (P<0.05) in broilers. The β-GOS supplementation showed no significant main effect on gene expression. In addition, there were also no interaction effects of β-GOS and methionine on the expression of all the genes (Fig. 2).

We also find out the correlations between the pH ($pH_{15min}$ and $pH_{24hrs}$) and the rate of ΔpH with the muscle degradation genes (MAFbx and MuRF1). We observed a pronounced association of the breast meat pH ($pH_{15min}$) with MuRF1 (P<0.001) and MAFbx (P=0.098) expression. However, there was no significant correlation between the muscle degradation genes or $pH_{24hrs}$ and ΔpH. Also, no significant correlation was observed between pH ($pH_{15min}$, $pH_{24hrs}$, and ΔpH) and MyoD as well as M-CK genes (Table 4).

**Table 1:** Primer sequences for gene expression analysis with RT-PCR

| Name of Gene | Primer | Sequence (5'-3') | Annealing Temperature (°C) |
|---|---|---|---|
| MAFbx | Forward | AGGCCGCAGTGTGTTGTTCT | 60 |
| | Reverse | GTGTGAATGGCTGGTTGCAT | |
| MuRF1 | Forward | GCCAAGCAGCTCATTAAAACG | 60 |
| | Reverse | CATGTTCTCATAGCCTTGCTCAAT | |
| MyoD | Froward | CAACAGCAGTGGTGT GACAGAT | 56 |
| | Reverse | CAAAGCAACTCTTATTTACAATTATACA | |
| M-CK | Forward | CGGAGCACCTGGGTTACATC | 60 |
| | Reverse | GGG GTGCTGGCTGAGTTTG | |
| GAPDH | Forward | CGATCTGAACTACATGGTTTACATGTT | 60 |
| | Reverse | CCCGTTCTCAGCCTTGACA | |

MAFbx: Muscle atrophy F-box; MuRF1: Muscle RING-finger 1; MyoD: Myogenic differentiator; M-CK: Muscle creatine kinase; GAPDH: Housekeeping gene.

**Table 2:** Effects of β-GOS and methionine co-supplementation on the meat quality attributes in broilers.

| Parameter | β-GOS | | | Methionine | | P-Value | | |
|---|---|---|---|---|---|---|---|---|
| | 0% | 0.2% | 0.5% | 0.5% | 1.0% | β-GOS | Methionine | β-GOS × Methionine |
| $pH_{15min}$ | 6.63±0.06[a] | 6.39±0.06[b] | 6.36±0.06[b] | 6.45±0.05 | 6.47±0.05 | 0.004 | 0.797 | 0.051 |
| $pH_{24hrs}$ | 5.98±0.06 | 5.97±0.06 | 5.90±0.06 | 5.95±0.05 | 5.95±0.05 | 0.611 | 0.929 | 0.891 |
| ΔpH | 0.77±0.07[a] | 0.47±0.07[b] | 0.45±0.07[b] | 0.54±0.06 | 0.59±0.06 | 0.005 | 0.555 | 0.141 |
| Drip Loss (%) | 7.21±1.17 | 7.42±1.17 | 9.45±1.17 | 5.90±0.95[b] | 10.1±0.95[a] | 0.331 | 0.003 | 0.289 |
| Cooking Loss (%) | 28.1±1.62[a] | 20.9±1.62[b] | 20.8±1.62[b] | 23.8±1.32 | 22.8±1.32 | 0.002 | 0.614 | 0.183 |
| MFD (mm) | 0.70±0.04 | 0.77±0.04 | 0.71±0.04 | 0.74±0.03 | 0.72±0.03 | 0.440 | 0.717 | 0.179 |
| MFbD (µm) | 42.0±1.92[a] | 34.0±2.07[b] | 34.3±2.07[b] | 35.7±1.66 | 37.9±1.74 | 0.021 | 0.387 | 0.558 |
| MFbDe (n/mm$^2$) | 342±23.8[b] | 446±23.8[a] | 511±23.8[a] | 419±19.4 | 447±19.4 | 0.001 | 0.331 | 0.980 |

Data presented as mean ± SE (n =12 birds per group); [a-b]Different superscripts within a row indicate significant differences; pH15min, pH24hrs mean pH measured at 15 minutes and 24 hours after slaughter respectively; ΔpH: Rate of pH change ( pH15min - pH24hrs); β-GOS: β-galacto-oligosaccharides; MFD: Muscle fascicle diameter; MFbD: Muscle fiber diameter; MFbDe: Muscle fiber density.

**Table 3:** Effects of β-GOS and methionine co-supplementation on the breast muscle MDA and catalase levels in broilers

| Parameter | β-GOS | | | Methionine | | P-Value | | |
|---|---|---|---|---|---|---|---|---|
| | 0% | 0.2% | 0.5% | 0.5% | 1.0% | β-GOS | Methionine | β-GOS × Methionine |
| MDA (µmol/L) | 1.16±0.05 | 1.04±0.05 | 1.09±0.05 | 1.12±0.04 | 1.08±0.04 | 0.193 | 0.395 | 0.378 |
| Catalase (KU/L) | 11.4±0.53[b] | 12.5±0.54[b] | 14.5±0.53[a] | 12.8±0.43 | 12.7±0.44 | 0.001 | 0.891 | 0.171 |

Data presented as mean ± SE (n =12 birds per group); [a-b]Different superscripts within a row indicate significant differences; β-GOS: β-galacto-oligosaccharides; MDA: Malondialdehyde.

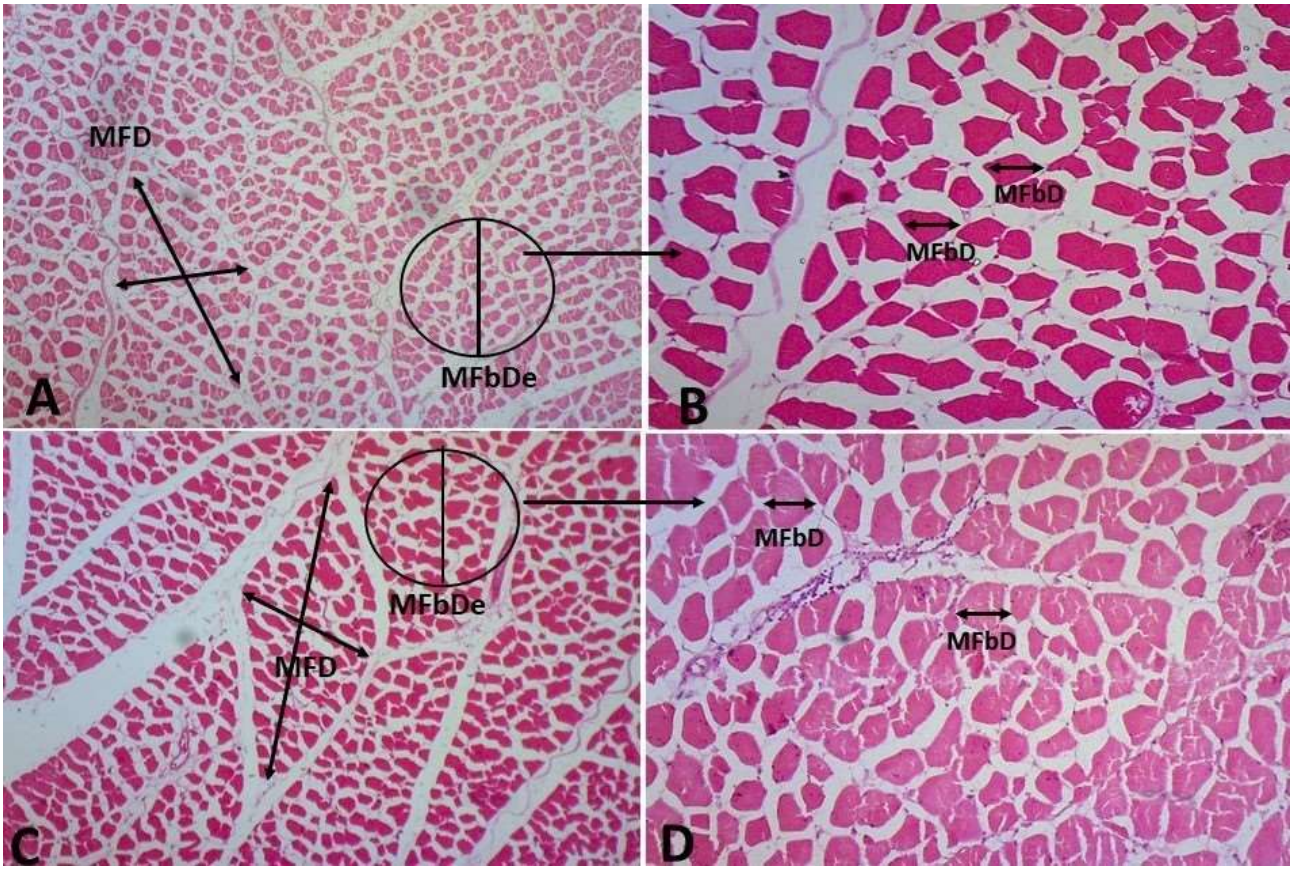


**Fig. 1:** Histomicrograph of broilers' breast muscle stained with H and E stain. Images **A** and **C** describe the measurements of **MFbDe** (muscle fiber density) and **MFD** (muscle fascicle diameter) in broilers' breast muscles at 4X. Images **B** and **D** show the **MFbD (**muscle fiber diameter) in broilers' breast muscles at 10X.

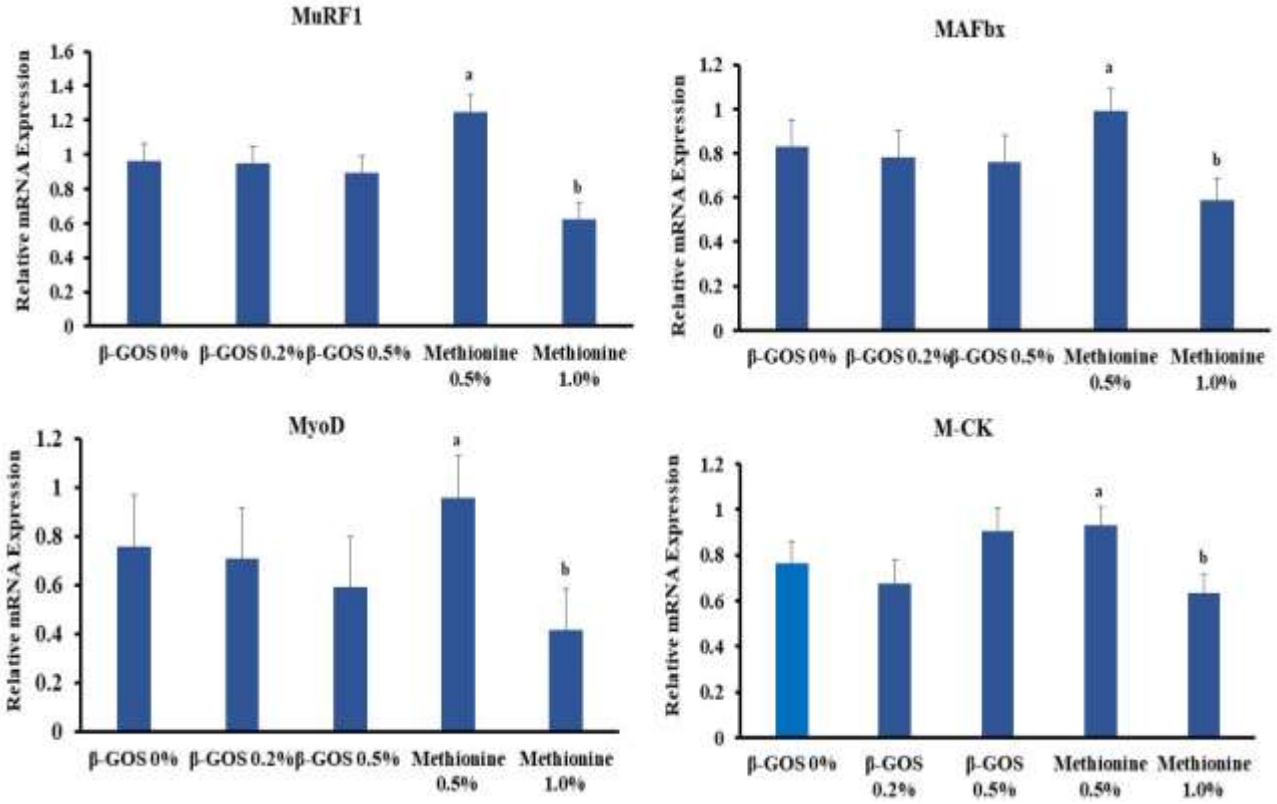


**Fig. 2:** Effects of β-GOS and methionine co-supplementation on relative gene expression ($2^{-\Delta\Delta CT}$). Data are presented as mean ± SEM (n = 12 birds per group). [a-b]Different superscripts within a row indicate significant differences. β-GOS: β-galacto-oligosaccharides; MAFbx: Muscle atrophy F-box; MuRF1: Muscle RING-finger 1; MyoD: Myogenic differentiator; M-CK: Muscle creatine kinase.

**Table 4:** Correlation coefficients (R) of the meat pH with various genes in the breast muscle of broilers.

| Gene | | $pH_{15min}$ | $pH_{24hrs}$ | ΔpH |
|---|---|---|---|---|
| MAFbx | *R* | 0.308 | 0.044 | -0.168 |
| | *p* | 0.098 | 0.816 | 0.375 |
| MuRF1 | *R* | 0.739 | 0.140 | -0.186 |
| | *P* | <0.001 | 0.476 | 0.343 |
| MyoD | *R* | 0.231 | 0.063 | -0.122 |
| | *P* | 0.219 | 0.742 | 0.521 |
| M-CK | *R* | -0.172 | -0.152 | -0.065 |
| | *P* | 0.363 | 0.422 | 0.734 |

pH15min, pH24hrs represent pH measured at 15 minutes and 24 hours post-slaughtered respectively; ΔpH: rate of pH change; MAFbx: Muscle atrophy F-box; MuRF1: Muscle RING-finger 1; MyoD: Myoblast determination protein 1; M-CK: Muscle creatine kinase; $p<0.05$.

## DISCUSSION

To the best of our knowledge, we are reporting for the first time the response of broilers following the co-supplementation of β-GOS and methionine on the selected meat quality attributes, meat microarchitecture, and the expressions of breast muscle genes.

The lower pH causes protein denaturation that results in water loss from meat (Mir *et al.,* 2017). We found a tendency of interaction (P=0.051) between β-GOS and methionine for the $pH_{15min}$ of meat, being significantly lower in the β-GOS-fed birds compared to non-fed birds. pH is the function of muscle glycogen and the rate at which it is converted into lactic acid (Mir *et al.,* 2017). Based on meat color and pH, meat is classified into three categories: pale soft, and exudative (PSE) meat, normal meat, and dark, firm, and dry meat (DFD) (Kralik *et al.,* 2014; Yang *et al.,* 2021). In the literature, the ultimate pH ($pH_{24hrs}$) has been used for the classification of meat into the PSE or DFD categories. However, Ristic and Klaus (2010), proposed that the initial pH measured 15 minutes after slaughtering can be used for the classification of meat and suggested the initial breast meat $pH_{15min}$ of >5.8 for "PSE", 5.9-6.2 for "normal", and above 6.3 for "DFD". In our study, the birds fed with a diet of 0% β-GOS had 6.63 $pH_{15min}$, which is in the DFD range, indicating deterioration in meat quality. Interestingly, GOS supplementation lowered the meat $pH_{15min}$ to acceptable ranges for normal meat while using the same classification criteria (Kralik *et al.,* 2014). Our findings are consistent with the results of Sang-Oh and Byung-Sung (2011), who reported that supplementing broilers with inulin (prebiotic) lowered the pH of the meat. In our study, the GOS supplementation prevented the rapid decline in pH compared with the non-supplemented birds. A rapid fall in the pH observed in the breast meat of 0%-β-GOS fed birds might have resulted in protein-fat denaturation and consequently reduced the muscle protein contents (Ma *et al.,* 2011). But the sustainability of meat pH was improved when the birds were on a diet supplemented with β-GOS. This positive effect of β-GOS might be due to the production of volatile fatty acids in the gut which serve as an energy source and slow down the process of accelerated glycolysis (Ma *et al.,* 2021), and also enhance the antioxidant property of β-GOS as observed in the current experiment.

Drip loss and cooking loss are the key indicators of the water-holding capacity of meat. Water loss reduces the meat's nutritional value as nutrients are believed to be lost in the exudate, making the meat less tender and worst in flavor (Pelicano *et al.,* 2003). Presently, we found that cooking loss was significantly reduced by β-GOS supplementation, which is in accordance with the findings of Cheng *et al.,* (2018), who recorded similar results in broilers fed a mannan-oligosaccharides-supplemented diet. However, some other studies showed that mannan-oligosaccharides and synbiotic supplementation had no effect on drip loss and cooking loss in broilers (Cheng *et al.,* 2017; Nisar *et al.,* 2021). The reduction in cooking loss might be attributed due to the ability of β-GOS that significantly attenuated the rapid fall of pH in meat as recorded in the current study.

Drip loss was significantly higher in the birds fed a diet containing 1.0% methionine compared to the birds fed 0.5% methionine. Like our study, Albrecht and co-workers also reported higher drip loss with increasing dietary methionine concentration in broilers (Albrecht *et al.,* 2017). The higher drip loss following methionine supplementation in our study might be due to various factors like rapid growth, intense genetic selection, rapid muscle growth, slaughtering, and processing conditions (Petracci and Cavani 2012).

In our study, the β-GOS supplementation increased (P<0.01) the muscle fibers density (MFbDe) with a concomitant decrease (P<0.05) in the thickness of muscle fiber. The thinner muscle fibers with more density are generally considered an indicator of meat fiberillarity and tenderness (Maiorano *et al.,* 2012; Dankowiakowska *et al.,* 2019). Previously, it has been found that muscle fiber density of pectoral muscle was slightly higher in birds injected in-ovo with either GOS or inulin compared to the control (Dankowiakowska *et al.,* 2019). Muscle fiber density has been considered the main determinant of muscle weight that ultimately influences the bird's body weight (Stasiak *et al.,* 2021). These outcomes may be correlated to our previously published data, (Ahmad *et al.,* 2022), in which 0.2% β-GOS positively affected the body weight and feed intake of broilers. This could be due to better nutrient utilization by GOS through increment in the villus surface area and enhanced beneficial intestinal microflora.

In the current study, the muscle catalase activity was higher in the 0.5% β-GOS supplemented birds. There is a dearth of information regarding the protective effects of GOS against oxidative stress in broilers. The previous study demonstrated the higher antioxidant capacity of the breast and thigh muscles in broilers supplemented with mannan-oligosaccharides and *Lactobacillus acidophilus* (Dev *et al.,* 2020). In another study, the antioxidant potential was improved with GOS supplementation in suckling pigs challenged with lipopolysaccharides (Tian *et al.,* 2022). The apparent higher antioxidant potential of GOS might be due to its bifidogenic nature as *Bifidobacterium animalis* has been suggestive to increase catalase activity by enhancing the production of ROS-inhibiting enzymes and upregulation of antioxidant signaling pathways to counter oxidative stress (Vitheejongjaroen *et al.,* 2022).

In the present study, the 1.0% methionine supplementation downregulated MURF1 and MAFbx expression, while MyoD and M-CK were upregulated in the 0.5% methionine-fed birds. The MURF1 and MAFbx, are the two major components of the proteasomal protein degradation pathway. MyoD is a muscle-specific

transcription factor, while, M-CK is a key enzyme in muscle energy metabolism (Li *et al.,* 2011). Similar results regarding the methionine-induced reduction in MAFbx expression in cisplatin-treated rats were reported previously (Wu *et al.,* 2019). Methionine stimulates the insulin-like growth factor-I (IGF-I) pathway in avian muscle fibroblasts which in turn inhibits the expression of MAFbx and MuRF1 and improves breast muscle growth (Wen *et al.,* 2014). Furthermore, methionine inclusion decreases the myostatin mRNA levels and increases the expression of myogenic regulatory factors and, hence, improves muscle protein deposition (Wang *et al.,* 2021). Similar effects of methionine on MyoD expression were observed in the present study, which could also be linked with the higher body weights of the same birds as reported earlier (Ahmad *et al.,* 2022). Furthermore, we found a strong association between meat $pH_{15min}$ and MuRF1 ($r = 0.739$, $P<0.001$) expression in breast meat of broilers, suggesting that the muscle protein degradation increases as pH increases. However, additional research studies are warranted to elucidate the detailed mechanisms involved.

**Conclusion**s: β-GOS and methionine co-supplementation did not show synergetic effects on selected meat quality attributes in broilers. However, the addition of β-GOS alone in the basal diet partially improved the meat quality in terms of a decrease in $pH_{15min}$, ΔpH, along with higher antioxidant activity, lower cooking loss, and enhanced muscle microarchitecture. Moreover, methionine supplementation counters the muscle degradative changes in breast muscle by downregulating the expressions of MuRF1 and MAFbx genes in broilers.

**Acknowledgments:** This research was supported by Higher Education Commission Pakistan, under Indigenous PhD Fellowship Program Phase II, Batch II (213-63792-2AV2-180).

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Author's contribution:** SA, HR, MSY, and MAR conceptualized and planned the study. SA, SKT, KAM, MN, MR, and AK performed the experiment and analyzed samples. SA, HZ, HR, and MSY analyzed the data. SA, HR, MSY, MAR, HZ, and ZH interpreted the data and critically revised the manuscript for important intellectual content. All the authors approved the final version.

## REFERENCES

Ahmad S, Yousaf MS, Tahir SK, *et al.,* 2022. Effects of co-supplementation of β-galacto-oligosaccharides and methionine on production performance, blood metabolites and gut histomorphometry in broilers. Pak Vet J 42:173-78.

Albrecht A, Herbert U, Miskel D, *et al.,* 2017. Effect of methionine supplementation in chicken feed on the quality and shelf life of fresh poultry meat. Poult Sci 96:2853-61.

Ashraf S, Zaneb H, Masood S, *et al.,* 2017. Influence of β-galacto-oligosaccharide on growth performance and components of intestinal barrier in broilers during heat stress. S Afr J Anim Sci 47:616-25.

Cheng Y, Chen Y, Li X, *et al.,* 2017. Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality and muscular antioxidant capacity and mineral contents in broilers. J Sci Food Agric 97:3699-3705.

Cheng Y, Du M, Xu Q, *et al.,* 2018. Dietary mannan oligosaccharide improves growth performance, muscle oxidative status, and meat quality in broilers under cyclic heat stress. J Thermal Biol 75:106-11.

Dankowiakowska A, Bogucka J, Sobolewska A, *et al.,* 2019. Effects of in ovo injection of prebiotics and synbiotics on the productive performance and microstructural features of the superficial pectoral muscle in broiler chickens. Poult Sci 98:5157-65.

Del Vesco AP, Gasparino E, Oliveira Neto AR, *et al.,* 2013. Dietary methionine effects on IGF-I and GHR mRNA expression in broilers. Genet Mol Res 12:6414-23.

Dev K, Mir NA, Biswas A, *et al.,* 2020. Dietary synbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens. Anim Nutr 6:325-32.

Elsharkawy MS, Chen Y, Liu R, *et al.,* 2021. Paternal dietary methionine supplementation improves carcass traits and meat quality of chicken progeny. Animals 11:325.

Hadwan MH and Abed HN, 2016. Data supporting the spectrophotometric method for the estimation of catalase activity. Data Brief 6:194-99.

Honikel KO, 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49:447-57.

Kralik G, Djurkin I, Kralik Z, *et al.,* 2014. Quality indicators of broiler breast meat in relation to colour. Anim Sci Pap Rep 32:173-78.

Li Q, Li J, Lan H, *et al.,* 2011. Effects of fasting and refeeding on expression of MAFbx and MuRF1 in chick skeletal muscle. Sci China Life Sci 54:904-7.

Livak KJ and Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:402-8.

Ma J, Wang J, Mahfuz S, *et al.,* 2021. Supplementation of mixed organic acids improves growth performance, meat quality, gut morphology and volatile fatty acids of broiler chicken. Animals 11:3020.

Maiorano G, Stadnicka K, Tavaniello S, *et al.,* 2017. In ovo validation model to assess the efficacy of commercial prebiotics on broiler performance and oxidative stability of meat. Poult Sci 96:511-18.

Mir NA, Rafiq A, Kumar F, *et al.,* 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J Food Sci Technol 54:2997-3009.

Nisar H, Sharif M, Rahman MA, *et al.,* 2021. Effects of dietary supplementations of synbiotics on growth performance, carcass characteristics and nutrient digestibility of broiler chicken. Braz J Poult Sci 23:1-10.

Ohkawa H, Ohishi N and Yagi K, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351-8.

Pelicano ERL, De Souza PA, De Souza HBA, *et al.,* 2003. Effect of different probiotics on broiler carcass and meat quality. Braz J Poult Sci 5:207-14.

Petracci M and Cavani C, 2012. Muscle growth and poultry meat quality issues. Nutrients 4:1-12.

Pokoo-Aikins A, Timmons JR, Min BR, *et al.,* 2022. Effects of varying levels of dietary dl-methionine supplementation on breast meat quality of male and female broilers. Poultry 1:40-53.

Khan I, Zaneb H, Masood S, *et al.,* 2022. Supplemental selenium nanoparticles-loaded to chitosan improves meat quality, pectoral muscle histology, tibia bone morphometry and tissue mineral retention in broilers. Pak Vet J 42:236-40.

Rehman H, Rosenkranz C, Böhm J, *et al.,* 2007. Dietary inulin affects the morphology but not the sodium-dependent glucose and glutamine transport in the jejunum of broilers. Poult Sci 86:118-22.

Ristic M and Damme K, 2010. The meaning of pH-value for the meat quality of broilers – Influence of breed lines. Technol Mesa 51:120-23.

Sang-Oh P and Byung-Sung P, 2011. Influence of inuloprebiotic supplementation of the diets of broiler chickens on shelf-life and quality characteristics of meat. J Anim Vet Adv 10:1336-41.

Stasiak K, Slawinska A and Bogucka J, 2021. Effects of probiotics, prebiotics and synbiotics injected in ovo on the microstructure of the breast muscle in different chicken genotypes. Animals 11:2944.

Tavaniello S, Slawinska A, Prioriello D, *et al.,* 2020. Effect of galactooligosaccharides delivered in ovo on meat quality traits of broiler chickens exposed to heat stress. Poult Sci 99:612-19.

Tian S, Wang J, Gao R, *et al.,* 2022. Early-life galacto-oligosaccharides supplementation alleviates the small intestinal oxidative stress and

dysfunction of lipopolysaccharide-challenged suckling piglets. J Anim Sci Biotechnol 13:1-14.

Vitheejongjaroen P, Kasorn A, Puttarat N, *et al.,* 2022. Bifidobacterium animalis MSMC83 improves oxidative stress and gut microbiota in D-galactose-induced rats. Antioxidants 11:2146.

Wang Y, Liu Q, Quan H, *et al.,* 2021. Nutraceuticals in the Prevention and Treatment of the Muscle Atrophy. Nutrients 13:1914.

Wen C, Jiang X, Ding L, *et al.,* 2017. Effects of dietary methionine on growth performance, meat quality and oxidative status of breast muscle in fast-and slow-growing broilers. Poult Sci 96:1707-14.

Wen C, Wu P, Chen Y, *et al.,* 2014. Methionine improves the performance and breast muscle growth of broilers with lower hatching weight by altering the expression of genes associated with the insulin-like growth factor-I signalling pathway. Br J Nutr 111:201-6.

Wu CT, Liao JM, Ko JL, *et al.,* 2019. D-methionine ameliorates cisplatin-induced muscle atrophy via inhibition of muscle degradation pathway. Integr Cancer Thera 18:1-15.

Yang Y, Wang W, Zhuang H, *et al.,* 2021. Prediction of quality traits and grades of intact chicken breast fillets by hyperspectral imaging. Br Poult Sci 62:46-52.

Yousaf MS, Ahmad I, Ashraf K, *et al*., 2016. Comparative effects of different dietary concentrations of β-galacto-oligosaccharides on growth performance, feed conversion efficiency and organs development in broilers. J Anim Plant Sci 26:1603-8.

Yousaf MS, Ahmad I, Ashraf K, *et al.,* 2017. Comparative effects of different dietary concentrations of β-galacto-oligosaccharides on serum biochemical metabolites, selected caecel microbiota and immune response in broilers. J Anim Plant Sci 27:98-105.