Effect of Prebiotics from Mannan Oligosaccharides and Fructo-oligosaccharides on Physio-biochemical Indices, Antioxidant and Oxidative Stability of Broiler Chicken Meat

Avishek Biswas (drbiswas007@rediffmail.com)
Central Avian Research Institute
Namit Mohan
Central Avian Research Institute
Kapil Dev
Central Avian Research Institute
N A Mir
Central Avian Research Institute
Ashok Kumar Tiwari
Central Avian Research Institute

Research Article

Keywords: lipid oxidation, mannan oligosaccharide, antioxidant activity, fructo oligosaccharide, broiler meat

Posted Date: March 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-275707/v1

Effect of prebiotics from mannan oligosaccharides and fructo-oligosaccharides on physio-biochemical indices, antioxidant and oxidative stability of broiler chicken meat'

Avishek Biswas[1]*, Namit Mohan[1], Kapil Dev[1], N.A Mir[1] and Ashok Kumar Tiwari[1]

[1]Avian Nutrition and Feed Technology Division,
ICAR-Central Avian Research Institute; Izatnagar, Bareilly, Uttar Pradesh 243122, India

***Corresponding Author**: Avishek Biswas
Tel: +91-581-2303223, Fax: +91-581-2301321,
E-mail: drbiswas007@rediffmail.com

ORCID
Avishek Biswas
https://orcid.org/0000-0002-1439-2477

Running title: Prebiotics and chicken meat quality

Antibiotics have revolutionized the intensive poultry production system as a feed additive by promoting growth, production and meat quality through improving gut health and reduction of sub-clinical infections during last five decades. However, currently, the usage of antibiotics in poultry production is under severe scientific and public scrutiny, because antibiotic growth promoter (AGP) has been linked to the possible development of antibiotic-resistant pathogens, which may pose a threat to human health. After European Union ban on in feed antibiotics as growth promoter in poultry, since 2006, prebiotics offer a potential substitute to in feed antibiotics. In this effort, the objective of this present study was to investigate the potentiality of prebiotics (mannan oligosaccharides-MOS and fructo-oligosaccharides-FOS) in replacement of antibiotic growth promoter and their relationship with physio-biochemical indices, antioxidant and oxidative stability and carcass traits of broiler chickens meat. 240 day-old broiler chicks (1 d) of uniform body weight were divided

into 30 replicate groups having 8 birds in each. Six corn based dietary treatments were formulated *viz.* T_1 (control diet), T_2 (T_1 + Bacitracin methylene di-salicylate @ 20 mg/kg diet), T_3 (T_1 + 0.1% MOS), T_4 (T_1 + 0.2% MOS), T_5 (T_1 + 0.1% FOS), and T_6 (T_1 + 0.2% FOS). Significant ($P<0.05$) increase in cut up part yields (%) and reduction in cholesterol and fat content in T_4 (0.2 % MOS) group. The water holding capacity (WHC) and extract release volume (ERV) were increase ($P<0.05$) in 0.1 or 0.2 % MOS supplemented group. DPPH (1, 1-diphenyl-2-picrylhydrazy) was higher ($P<0.05$) and lipid oxidation (free fatty acid and thio-barbituric acid reactive substances) was lower ($P<0.05$) in T_4 group. The standard plate count (SPC), staphylococcus and coliform counts were decreased ($P<0.05$) in T_3 or T_4 group. Thus, it can be concluded that mannan oligosaccharides (MOS) may be incorporated at 0.2% level in diet for improved physio-biochemical indices, antioxidant and oxidative stability and carcass characteristics of broiler chickens meat and it may be suitable replacer of antibiotic growth promoter.

Keywords: lipid oxidation, mannan oligosaccharide, antioxidant activity, fructo oligosaccharide, broiler meat

1. Introduction

In poultry production, 70-75% of production cost is constituted by feed cost which is increasing constantly with time. There can be considerable reduction of feed cost if it is formulated with precise supply of nutrients. The use of antibiotics growth promoters (AGPs) as feed additives in intensive poultry production system have resulted in significant improvement of growth and feed efficiency of birds by improving gut health and reducing sub-clinical infections. Antibiotics prevent the thickening of intestine ensuring better nutrient absorption as well as spare the critical nutrients by reducing the competition between host and microbes. Currently, majority of the scientific community as well as public is highly cynical about the application of AGPs in poultry feed at sub-therapeutic doses, because the

AGPs have been strongly linked to the possible development of antibiotic-resistant pathogens, which may pose a threat to human health[1]. Microbial meat quality is another important issue that has to be considered very seriously as the entero-pathogens like *E. coli* and *staphylococci* possess public health hazard and food borne intoxication[2]. World health organization (WHO) reported that about 76 million cases of food-borne illness occur in each year, resulting in an estimated 3, 25,000 hospitalizations and 5000 deaths. Despite the efforts, the incidence of human infections from entero-pathogens such as *Salmonella*, *E. coli* and *Campylobacter* have increased over the past 30 years. Contamination of poultry meat and by products occurs as a result of exposure of the animal carcass to gut pathogens during or after slaughter[3].

Natural feed additives, such as 'prebiotics' have a potential to reduce enteric pathogen load in poultry and the subsequent contamination of poultry products[4]. Prebiotics are defined as non-digestible ingredients which selectively stimulate the growth and/or the activity of a limited number of beneficial intestinal bacteria[5]. A number of studies have supported the beneficial effects of using prebiotics in improving the animal health and production [6, 7]. However, the evaluation of beneficial effects of these feed additives must not be limited to growth performance only, but should also include the quality and safety aspects of meat. A number of research results are available on this subject, but there is no consensus between these results. Some researchers deliberate that feeding of prebiotics have been useful to improve the meat and carcass quality[8], whereas, others have refuted such results[9].

Thus, the objective of this experiment was to investigate the effect prebiotics (mannan oligosaccharides-MOS and fructo-oligosaccharides-FOS) in replacement of antibiotic growth promoter and their relationship with physio-biochemical indices, antioxidant and oxidative stability and carcass characteristics of broiler chickens meat.

2. Material and methods

83 **2.1.** Ethical Approval

84 All the procedures and protocols followed in the study were performed as per the guidelines
85 of the Institute Animal Ethics Committee (IAEC) and CPCSEA-17 approval (No.
86 CARI/IAEC/ 17/11)' which has been established under the 'Prevention of Cruelty to Animals
87 Act 1960' of Indian Penal Code.

88 **2.2. Experimental population**

89 A total of 240 day-old straight-run (sex ratio ≈ 1) commercial broiler chicken of uniform
90 body weight were used in this study. The birds were divided randomly into 30 replicate
91 groups having 8 birds in each with equal number of males and females, distinguished by vent
92 sexing method. The birds were reared under uniform standard managemental conditions in
93 electrically heated battery brooders (12 ft^2 for 8 birds i.e., 1.5 ft^2 per bird) and birds were
94 vaccinated following the routine vaccination schedule of our experimental farm. The birds
95 were provided 24 h light for first three days followed by a decrease of 1 h per day till it
96 reached 18 h light period which was continued till the end of trial. The initial cage
97 temperature was 95 °F which was reduced by 5 °F every week to provide thermo-comfort
98 environment to the birds.

99 **2.3. Experimental diets**

100 Six iso-caloric and iso-nitrogenous corn-soya based dietary treatments were formulated *viz.*
101 T_1 (control diet), T_2 (T_1 + Bacitracin methylene di-salicylate @ 20 mg/kg diet), T_3 (T_1 + 0.1%
102 MOS), T_4 (T_1 + 0.2% MOS), T_5 (T_1 + 0.1% FOS), and T_6 (T_1 + 0.2% FOS). Each of the
103 treatment was assigned five replicate groups of birds. The birds were provided *ad libitum*
104 respective feed and fresh water throughout the feeding trial of 42 days.

105 **2.4. Carcass characteristics**

106 At the end of 42 days experimental period 15 birds from each treatment (three birds per
107 replicate group) were electrically stunned (200V applied for 3s) and slaughtered by

exsanguination after 12 h of fasting with *ad libitum* drinking water. The carcass characteristics, cut up parts, and relative weight of organs (spleen, heart, liver, bursa of Fabricius and thymus) were determined.

2.5. Collection of sample

The breast and thigh meat samples were collected individually from each slaughtered bird for the study of physio-biochemical, oxidative stability, and microbial characteristics.

2.6. Physio-biochemical indices

Fat content (percentage, dry basis) of meat was determined by refluxing 2 g dried meat sample in 150 ml petroleum ether in Soxhlet extraction apparatus for 6 h at 60 °C[10]. For cholesterol estimation about 1 g meat sample was extracted in 15 ml chloroform methanol mixture (2:1) and the concentration of cholesterol in the extract was determined by spectrophotometer at wavelength of 560 nm[11]. The pH of meat was measured with the help of digital pH meter by blending 5 g meat sample with 25 ml distilled water for two minutes [12]. For the estimation of purge loss/drip loss, the frozen meat samples were weighed and recorded as the initial weight (W1). The weighed samples were placed into polyethylene bags, labelled, and stored hanging at 4^0 C for 24 h. The meat samples were weighed again and final weight (W2) was recorded. Drip loss was calculated as shown in the equation below:

$$\text{Drip loss (\%)} = [(W1 - W2) / W1] \times 100.$$

To determine the extract release volume (ERV) of meat samples, 15 g samples were blended with 60 ml phosphate buffer solution (0.05 M & pH 5.8) for two minutes and the homogenate was filtered through Whatman filter paper No. 1 for a fixed time period of 15 minutes to the filtrate measured as ERV[13]. Water holding capacity (WHC) of meat samples was determined by mixing 10 g minced meat sample in 15 ml of 0.6 M NaCl for 2 minutes followed by refrigerated (4 °C) holding for 15 minutes. The slurry is then shaken, centrifuged at 5000 RPM for 15 minutes, the supernatant fluid was decanted and measured[14].

WHC (%) = [(vol. of NaCl added – vol. of supernatant) / weight of sample] x100.

2.7. Lipid peroxidation parameters

Thio-barbituric acid reactive substance (TBARS) value: About 5 g meat sample was extracted in 12.5 ml 20% TCA (made in 2 M orthophosphoric acid) solution for 2 minutes and the slurry was mixed with 12.5 ml cold distilled water followed by filtration through Whatman paper No. 1. Then 3 ml of filtrate was mixed with 3 ml of TBA reagent (0.005 M), mixture was kept in dark cabinet for 16 h and absorbance (O.D) was measured by spectrophotometer (UV/VIS, Varian) at fixed wavelength of 532 nm against the blank made by mixing of 3 ml of 10% TCA and 3 ml of TBA reagent[15]. TBARS value was calculated as mg malonaldehyde (MDA) per Kg of sample by multiplying O.D value with K-factor of 5.2.

Free fatty acid value and Peroxide value: About 5 g meat sample was blended with 30 ml chloroform for 2 minutes in presence of anhydrous sodium sulphate powder followed by filtration into conical flask through No. 1 Whatman paper[16]. For free fatty acid value about 2-3 drops of 0.2% phenolphthalein indicator was added to the chloroform extract followed by titration with 0.1 N alcoholic potassium hydroxide to get the pink colour end point. For peroxide value 30 ml of glacial acetic was added to 25 ml of chloroform extract, then 2 ml potassium iodide solution was added, and the mixture was allowed to stand for 2 minutes with occasional shaking. Then, 100 ml distilled water and 2 ml fresh 1% starch solution were added to the mixture following titration with 0.1 N sodium thiosulphate till the end point was reached (non-aqueous layer turned colourless). The calculations were made as follows:

Free fatty acid (%) = [(0.1 x vol. of KOH consumed x 0.282) / sample weight] x100.

Peroxide value (meq/kg) = [(0.1 x vol. of sodium thiosulphate consumed) / sample weight] x1000.

2.8. Antioxidant parameters

About 5 g meat sample was triturated in 20 ml ethanol for 2 minutes followed by filteration through Whatman paper No. 42. For $ABTS^+$ (2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay 2 ml of ABTS working solution (7 mM) was added to 1 ml filtrate and absorbency was measured by spectrophotometer (UV/VIS, Varian) at fixed wavelength of 734 nm after 20 minutes (At_{20})[17]. For DDPH (1, 1-diphenyl-2- picrylhydrazyl) assay 1 ml filtrate was mixed with 1 ml 0.1 M Tris-HCl buffer (pH 7.4) and 1 ml DPPH reagent (250 μM). The absorbency was measured immediately (At_0) and after 20 minutes (At_{20}) by spectrophotometer (UV/VIS, Varian) at fixed wavelength of 517 nm[18]. The calculations were made as follows:

$$\text{ABTS activity (\% inhibition)} = [(0.7 - At_{20}) / 0.7] \times 100.$$

$$\text{DPPH activity (\% inhibition)} = 100 - (At_{20} / At_0) \times 100.$$

2.9. Estimation of microbial count

The microbial load of the meat samples were estimated in terms of specific plate count (SPC), coliform count, and staphylococcus count. About 1 g sample was homogenized with 10 ml of 0.1% peptone water (Hi-media) with the aid of sterile pestle and mortar under aseptic condition to give a 10:1 initial dilution. The homogenate was used for the preparation of tenfold serial dilution up to 10^6:1 with 0.1% peptone water in sterile test tubes. One ml aliquot of each dilution was placed in identified sterile petridishes aseptically. About 12-15 ml of sterile molten and cooled (45°C) specified agar (Himedia) was poured on each petridish and mixed gently. After setting, the plates were incubated at 37°C for 48 hours and colonies were counted using a Quebec colony counter. The counts were multiplied by the respective dilution and calculated per gram of sample as $\log_{10}$ cfu.

2.10. Statistical Analysis

For the data analysis each bird was taken as an experimental unit. The data were analysed by one way ANOVA using the General Linear Model procedure (IBM SPSS softeware-20). The

Tukey post-hoc analysis was done to test the significant mean differences between the treatment groups with significance level defined at $P<0.05$.

3. Results

3.1. Carcass traits

The effect of prebiotics on carcass characteristics and cut-up parts are presented in table-1 and 2. No significant ($P>0.05$) difference was observed in dressed and eviscerated yields, whereas, significantly ($P<0.05$) higher thigh, breast, back, and drumstick weights (% of live weight) were observed in T_4 (0.2% MOS) group followed by statistically similar T_3 (0.1% MOS) compared to control, antibiotic, and other prebiotic supplemented groups (table 2). No significant ($P>0.05$) differences were observed in neck, wing, and organ (heart, liver and gizzard) weights among the dietary supplemented groups.

3.2. Physico-biochemical indices

The results of various physico-biochemical parameters as affected by feeding of prebiotics in diet to broiler chicken are shown in table 3. The cholesterol and fat content of the meat were significantly ($P<0.05$) lower in birds fed 0.2% MOS (T_4 group) which was statistically similar to other MOS and FOS supplemented birds. The cholesterol and fat content of meat was higher in birds fed control diet (T_1 group) or BMD supplemented diet (T_2 group) which was statistically similar to T_3, T_5, and T_6 groups. The pH and drip loss (%) of chicken meat were not significantly ($P>0.05$) influenced by dietary treatments. Significantly higher ($P<0.05$) WHC and ERV of chicken meat was observed in birds fed 0.2% MOS (T_4 group) or 0.1% MOS (T_3 group) which were statistically similar to WHC and ERV of meat from FOS fed birds (T_5 and T_6 groups). The meat from birds fed control diet (T_1 group) or BMD supplemented diet (T_2 diet) revealed lower WHC and ERV values which did not differ significantly from FOS supplemented birds.

3.3. Lipid oxidation parameters

The lipid peroxidation parameters are given in table 4. The TBARS and free fatty acid (FFA) values revealed significant ($P<0.05$) differences among the dietary treatments. The TBARS and FFA values of broiler chicken meat were lower in birds fed 0.2% MOS (T_4 group) which did not differ significantly from other MOS, FOS, and BMD supplemented birds. The higher values were observed in control diet fed birds which were statistically to 0.1% MOS, BMD, and FOS supplemented birds. The peroxide values of chicken meat did not show any significant dietary effect.

3.4. Antioxidant Parameters

The results of antioxidant parameters affected by prebiotic supplementation in broiler chicken are given in table 5. No significant differences were observed in ABTS values of breast and thigh meat among the dietary treatments. However, DPPH values of chicken breast and thigh meat were significantly ($P<0.05$) higher in birds fed 0.2% MOS (T_4 group) compared to control and BMD supplemented birds. But other MOS and FOS supplemented birds resulted in DPPH values similar to that of T_4 group birds.

3.5. Microbial quality

The results of microbial load of chicken meat as influenced by prebiotic supplementation are given in Table 6. Significant reduction in SPC, coliform, and staphylococcus counts were observed in meat of birds supplemented with 0.2% MOS (T^4) and 0.1% MOS (T^3) compared to control diet and antibiotic fed birds. The MOS and FOS supplemented birds did not differ significantly from each other.

4. Discussion

4.1. Carcass traits

Similar to the results of present study, Saleh et al.[19] reported that carcass and cut-up parts yields were significantly higher in chicken fed prebiotic containing diet. However, in contrast to the present study Hidalgo et al.[20] reported no significant differences in breast, thigh, and

carcass yields after dietary inclusion of prebiotics. Whereas, Pelicano et al. [7] observed no significant effect of prebiotics on the cut-up parts of chicken carcass. Therefore, based on the results of present study it can be assumed that the application of prebiotics has a positive effect on muscle weight.

4.2. Physico-biochemical indices

The results of the present study are in line with the findings of Pilarski et al.[21], who reported that prebiotics caused a decrease in meat cholesterol concentration in comparison to the control and antibiotic treated group. In contrast, Salma et al.[22] reported that no significant difference was observed in cholesterol concentration after dietary inclusion of prebiotics. The results of the present study were in accordance with the findings of Khaksefidi and Rahimi[23], who observed that fat % of breast meat, was significantly lower in prebiotic supplemented chicken.

In the present study, the pH values were within the normal range and independent of dietary prebiotic supplementation. Similar to the results of the present study Mir et al.[3] did not find any significant effect of dietary prebiotic supplementation on the pH values of chicken meat. However, Mir et al.[12] confirms that the meat quality is influenced by pH changes which occurr during rigor mortis. Generally meat with high pH has high WHC, although the present study does not support this correlation. The results of present study are in line with Habibi-Najafi et al.[24], who reported that dietary supplementation of prebiotic increased the WHC of meat. On the other hand, Pelicano et al.[25] reported that dietary inclusion of prebiotic has no significant effect on WHC of meat during storage condition. It is remarkable to note that water loss reduces meat nutritional value because some nutrients may be lost in exudate resulting in meat becoming less tender and bad in flavour. Regarding ERV values in broiler chicken after the dietary inclusion of FOS and MOS, no such reports are available for comparing the results of this study.

4.3. Lipid oxidation parameters

The results of present study showed that prebiotic could inhibit both thigh and breast muscle lipid oxidation (MDA production) in broiler chicken, therefore protecting the peroxidation of labile PUFA enriched meat. The reduced shelf-life of meat occurs due to progressive oxidation and enzymatic hydrolysis of unsaturated fatty acid [26] FFA value is the measure of hydrolytic rancidity due to lipolytic enzyme activity of microbial and muscle origin resulting in accumulation of FFA which might impart undesirable flavour in foods[27]. The peroxide value test involves the measurement of peroxide and hydro peroxide formed during initial stage of lipid oxidation[12]. However, in contrast to the results of present study Konca et al.[28] reported that after the dietary inclusion of prebiotics, TBARS values were significantly increased. Furthermore, Ali[29] reported that dietary inclusion of prebiotics has no pivotal role in changing the TBARS activities in fresh as well as stored meat.

4.4. Antioxidant Parameters

The natural dietary antioxidant compounds of plant origin react with lipids and hydroxyl radicals and result into stable product. Simitzis et al.[30] reported that following absorption prebiotics have shown significant antioxidant activity in poultry meat after entering the systemic circulation. The lipid and cholesterol oxidation of broiler chicken meat was significantly reduced by dietary prebiotic supplementation in broiler chicken[31]. Inclusion of prebiotics in turkey diet increased the oxidation stability and retention of alpha tocopherol in the long term stored frozen turkey meat[32]. It is still unclear whether the dietary antioxidants consumed can be incorporated into fatty tissues in the same form as when the fat is stabilized *in-vitro*[33]. However in the present study, free radical inhibition percentage of thigh and breast meat of chicken fed 0.2% MOS was significantly greater than that of chicken fed control and antibiotic supplemented diet. These results indicate that antioxidant compounds from prebiotic prevented thigh and breast meat from oxidation.

4.5. Microbial quality

According to the hypothesis proposed by Chaeveerach et al.[34], the reduction in microbial load was due to production of different antimicrobial components by prebiotic which result in exclusion of common entero-pathogens and food spoilage organisms of broiler chicken. Though, the exact mechanism by which prebiotics might exert anti-microbial effects in broiler chicken meat remains unclear. Some of the proposed modes of actions are; maintaining a healthy balance of gut microflora, competitive exclusion and inhibition of microbial growth by lactic acid producing bacteria favoured by dietary prebiotics, enhancing gut immunity and integrity, improving digestive enzyme activities, digestion and neutralizing enterotoxins, etc.[35].

Conclusion

The results reported in this work indicate that 0.2% mannan oligosaccharides (MOS) could be used as natural growth promoter (NGP) to replace the antibiotic growth promoter (AGP) in improving the physio-biochemical, oxidative stability, and microbiological quality of broiler chicken meat. Subsequent the appropriate guidelines and protocols will ensure eventually limited the use of feed antibiotic for poultry production and the induction of NGP in animal derived food products i.e., meat which will reduce the risk to the public. This NGP could be popularized among the farmers as a feed additive in poultry diets for production of safe, clean, and green poultry meat for human consumption.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Acknowledgment

The study was funded by the Department of Biotechnology, Ministry of Science and Technology, Government of India with Grant No. BT/PR9724/AAQ/1/571/2013.

References

1. Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K., Glenn, J. & Morris. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *National Academy of Sciences of the United States of America*, 99, 6434–6439. DOI:10.1073/pnas.082188899 (2002).
2. Panisello, P. J., Rooney, R., Quantick, P. C. & Stanwell-Smith, R. Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. *International Journal of Food Microbiology*, 59, 221-234. DOI: 10.1016/s0168-1605(00) 00376-7 (2000).
3. Mir, N. A., Rafiq, A., Kumar, F., Singh, V. & Shukla, V. Determinants of broiler chicken meat quality and factors affecting them: a review. *Journal of Food Science and Technology*, 54(10), 2997-3009; DOI: 10.1007/s13197-017-2789-z (2017a).
4. Willis, W. L. & Reid, L. Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and Campylobacter jejuni presence. *Poultry Science*, 87(4), 606- 611. DOI:10.3382/ps.2006-00458 (2008).
5. Gibson, G. R., Probert, H. M., Van Loo, J. A. E. & Roberfroid, M. B. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutritional Research Review*, 17, 257-259. DOI: 10.1079/nrr200479 (2004).
6. Santin, E., Maiorka, A., Macari, M. Grecco, M., Okada, T. M. & Myasaka, A. M. Performance and intestinal mucosa development of broiler chickens fed diets containing Saccharomyces cerevisiae cell wall. *Journal of Applied Poultry Research* 10(3), 236-244. DOI: 10.1093/japr/10.3.236 (2001).
7. Pelicano, E. R. L., Souza, P. A, Souza, H. B. A., Oba, A., Boiago, M. M., Zeola, N. M. B. L., Scatolini, A. M., Bertanha, V. A. & Lima. T. M. A. Carcass and cut yields and

meat qualitative traits of broilers fed diets containing probiotics and prebiotics. *Brazilian Journal of Poultry Science*, 7, 169-175. (2005).

8. Santos, S. D. L. F., Donoghue, A. M., Farnell, M. B., Huff, G. R., Huff W. E. & Donoghue, D. J. Gastrointestinal maturation is accelerated in turkey poult's supplemented with a mannan-oligosaccharide yeast extract (*Alphamune*). *Poultry Science*, 86, 921-930. DOI:10.1093/ps/86.5.921 (2007).

9. Quadros, A. R .B., Kiefer, C., Ribeiro, N. L. C. & Zink, L. A. Características qualitativas da carne de suínos alimentados com rações contendo ou não probióticos. In: 38th *Reunião Annual da SBZ, Piracicaba Anais Piracicaba*, 794-795. (2001).

10. AOAC. Official methods of analysis (16th ed.). Arlington, VA: Association of Analytical Chemists, 1995.

11. Wybenga, D. R. & Pileggi, V. J. Estimation of cholesterol. Clinical Chemistry, 16: 980. (1970).

12. Mir, N. A., Tyagi Praveen, K., Biswas, A. K., Tyagi Pramod, K., Mandal, A. B., Sajad, A. S., Deo, C., Divya, S. & Verma, A. K. Impact of feeding chromium supplemented flaxseed based diet on fatty acid profile, oxidative stability and other functional properties of broiler chicken meat. *Journal of Food Science and Technology*, 54(12), 3899-3907 (2017b).

13. Jay, J. M. & Hollingshed, A. M. Two methods for determining extract release volume (erv) of fresh and spoiled beef and poultry meats. *Journal of Food Science* 55(5), 1475-1476· (2006).

14. Brondum, J., Munck, L., Henckel, P., Karlsson, A., Tornberg, E. & Soren, B. E. Prediction of water-holding capacity and composition of porcine meat by comparative spectroscopy. *Meat Science*, 55(2), 177-185; DOI: 10.1016/S0309-1740(99)00141-2. (2000).

15. Alam, Z. & Fareed, U. A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. *Journal of Analytical Methods in Chemistry*, Article ID 9412767, 5 pages; DOI:10.1155/2016/9412767. (2016).

16. Sallam, K.I., Ishioroshi M. & Samejima, K. Antioxidant and antimicrobial effects of garlic in chicken sausage. Lebensmittel-Wissenschaft Technologie (LWT), 37:849-855. (2004).

17. Shirwaikar, A., Prabhu, K. & Punitha, I. S. R. In-vitro antioxidant studies of Sphaeranthus indicus. *Indian Journal of Experimental Biology*, 44, 993-998. (2006).

18. Saleh, H., Golian, A., Kermanshahi, H. & Mirakzehi, M. T. Antioxidant status and thigh meat quality of broiler chickens fed diet supplemented with α-tocopherolacetate, pomegranate pomace and pomegranate pomace extract. *Italian Journal of Animal Science*, 17(2), 386-395, DOI: 10.1080/1828051X.2017.1362966. (2018)

19. Saleh, E. A., Watkins, S. E., Waldroup, A. L. & Waldroup, P. W. Effects of dietary nutrient density on performance and carcass quality of male broilers grown for further processing. *International Journal of Poultry Science*, 3, 1-10 (2004).

20. Hidalgo, M. A., Dozier, W. A., Davis, A. J. & Gordon, R.W. Live performance and meat yield responses of broilers to progressive concentrations of dietary energy maintained at a constant metabolizable energy-to-crude protein ratio. *Journal of Applied Poultry Research,* 13, 319-327, https://doi.org/10.1093/japr/13.2.319. (2004).

21. Pilarski, R., Bednarczyk, M., Lisowski, M., Rutkowski, A., Bernacki, Z., Wardeńska, M. & Gulewicz, K. Assessment of the effect of α-galactosides injected during embryogenesis on selected chicken traits. *Folia Biologica*. (Krakow), 53, 13-20. (2005).

381 22. Salma, U., Miah, A. G., Maki, T., Nishimura, M. & Tsujii, H. Effect of dietary
382 Rhodobacter capsulatus on cholesterol concentration and fatty acid composition in
383 broiler meat. *Poultry Science*, 86, 1920-1926. (2007).
384 23. Khaksefidi, A. & Rahimi, S. Effects of probiotic inclusion in the diet of broiler chickens
385 on performance, feed efficiency and carcass quality. *Asian Australasian Journal of*
386 *Animal Sciences*, 18, 1153-1156. (2005).
387 24. Habibi-Najafi, M. B., Fatemizadeh, S. S. & Tavakoli, M. Effect of fat percentage and
388 prebiotic composition on proteolysis, ace-inhibitory and antioxidant activity of
389 probiotic yogurt. *International Journal of Nutrition and Food Engineering*, 11(8), 622-
390 628. (2017).
391 25. Pelicano, E. R. L., Souza, P. A, Souza, H. B. A., Oba, A., Norkus, E. A., Kodawara, L.
392 M. & Lima, T. M. A. Effect of different probiotics on broiler carcass and meat quality.
393 *Brazilian Journal of Poultry Science*, 5, 207-214. (2003).
394 26. Nazemroaya, S., Sahari, M. A. & Rezaei M. Effect of frozen storage on fatty acid
395 composition and changes in lipid content of Scomberomorus commersoni and
396 Carcharhinus dussumieri. *Journal of Applied Ichthyology*, 25, 91-95. (2009).
397 27. Kim, H. W., Yan, F. F., Hu, J. Y., Cheng, H. W. & Kim, Y. H. B. Effects of probiotics
398 feeding on meat quality of chicken breast during post-mortem storage. *Poultry Science*,
399 95(6), 1457-1464. (2016).
400 28. Konca, Y., Kirkpinar, F. & Selim, M. Effects of Mannan-oligosaccharides and live yeast
401 in diets on the carcass, cut yields, meat composition and colour of finishing turkeys.
402 *Journal of Animal Sciences* 22, 550-556. (2009).
403 29. Ali, F. H. M. Probiotics feed supplement to improve quality of broiler chicken carcasses.
404 *World Journal of Dairy and Food Science*, 5, 93-99. (2010).

30. Simitzis, P. E., Bizelis, J. A., Deligeorgis, S. G. & Feggeros, K. Effect of early dietary experiences on the development of feeding preferences in semiintensive sheep farming systems-a brief note. *Applied Animal Behaviour Science*, 111, 391-395. DOI:10.1016/j.applanim.2007.06.010 (2008).

31. Botsoglou, N. A., Govaris, A., Botsoglou, E. N., Grigoropoulou S. H. & Papageorgiou, G. Antioxidant activity of dietary oregano essential oil and alpha-tocopherol acetate supplementation in long termfrozen stored turkey meat. *Journal of Agricultural and Food Chemistry*, 51, 2930-2936. DOI: 10.1021/jf021034o (2003).

32. Botsoglou, N. A., Florou-Paneri, P., Christaki, E., Fletouris, D. J. & Spais, A. B. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British Poultry Science,* 43, 223-230. DOI:10.1080/00071660120121436 (2002).

33. Cervato, G., Carabelli, M., Gervasio, S., Cittera, A., Cazzola R. & Cestaro, B. Antioxidant properties of oregano (origanum vulgare) leaf extracts. *Journal of Food Biochemistry*, 24, 453-465; DOI:10.1111/j.1745-4514.2000.tb00715.x (2000).

34. Chaveerach, P., Keuzenkamp, D. A., Lipman, L. J. & Van Knapen, F. Effect of organic acids in drinking water for young broilers on Campylobacter infection, volatile fatty acid production, gut microflora and histological cell changes. *Poultry Science,* 83(3), 330-334; DOI: 10.1093/ps/83.3.330 (2004).

35. Brisbin, J.T., Gong, J., Parvizi, P. & Sharif, S. Effects of lactobacilli on cytokine expression by chicken spleen and caecal tonsil cells. *Clinical and Vaccine Immunology*, 17, 1337-1343. DOI:10.1128/CVI.00143-10. (2010).

Table 1. Effects of dietary inclusion of prebiotics on carcass characteristics and organ weight (% of live weight) in broiler chickens (n = 15)

Group	Dietary treatments	Dressing yield	Eviscerated yield	Heart	Liver	Gizzard
T1	Control diet	71.34	65.45	0.50	2.25	2.02
T2	T1+ BMD@20 mg	70.23	65.74	0.49	2.17	1.95
T3	T1+ 0.1% MOS	72.34	67.82	0.54	2.32	2.08
T4	T1+ 0.2% MOS	72.24	65.66	0.55	2.26	2.01
T5	T1+ 0.1% FOS	71.32	65.68	0.53	2.23	1.95
T6	T1+ 0.1% FOS	71.16	65.56	0.51	2.21	1.98
SEM	-	3.25	2.32	0.001	0.002	0.002
P-value	-	NS	NS	NS	NS	NS

Mean values bearing the same superscript in a column did not differ significantly ($P < 0.05$).

435

Table 2. Effects of dietary inclusion of prebiotics on cut up parts (% of live weight) in broiler chickens (n = 15)

Group	Dietary treatments	Thigh	Breast	Back	Wings	Neck	Drumstick
T1	Control diet	9.82^{a}	16.54^{a}	17.44^{a}	7.94	4.62	10.10^{ab}
T2	T1+ BMD@20 mg	9.67^{a}	16.30^{a}	17.38^{a}	7.89	4.55	09.94^{a}
T3	T1+ 0.1% MOS	10.04^{b}	17.45^{ab}	18.20^{b}	8.07	4.70	10.84^{b}
T4	T1+ 0.2% MOS	10.45^{b}	18.50^{b}	18.24^{b}	8.04	4.64	10.60^{b}
T5	T1+ 0.1% FOS	9.91^{a}	17.10^{ab}	18.13^{b}	8.03	4.59	10.34^{ab}
T6	T1+ 0.1% FOS	9.99^{a}	16.95^{a}	18.05^{b}	7.99	4.50	10.21^{ab}
SEM	-	0.37	0.56	0.42	0.95	0.24	0.48
P-value	-	<0.05	<0.05	<0.05	NS	NS	<0.05

Mean values bearing the same superscript in a column did not differ significantly ($P < 0.05$).

436

437

438

439

440

441

442

443

444

Table 3. Effects of dietary inclusion of prebiotics on physio- biochemical characteristics of meat in broiler chickens (n = 15)

Group	Dietary treatments	pH	WHC[#] (%)	Drip Loss (%)	Cholesterol (mg/dl)	Fat (%)	ERV[$] (mL)
T1	Control diet	5.55	43.14^{a}	2.60	55.08^{b}	3.62^{b}	13.05^{a}
T2	T1+ BMD*@20 mg/kg	5.56	43.32^{a}	2.52	55.01^{b}	3.55^{b}	13.24^{a}
T3	T1+ 0.1% MOS**	5.59	46.53^{b}	2.39	52.10^{ab}	3.25^{ab}	15.85^{b}
T4	T1+ 0.2% MOS**	5.57	47.17^{b}	2.45	49.08^{a}	2.78^{a}	16.08^{b}
T5	T1+ 0.1% FOS***	5.60	44.84^{ab}	2.39	53.32^{ab}	3.26^{ab}	14.37^{ab}
T6	T1+ 0.1% FOS***	5.58	44.92^{ab}	2.47	52.76^{ab}	3.30^{ab}	14.44^{ab}
SEM	-	0.07	4.15	0.05	3.16	0.45	1.67
P-value	-	NS	<0.05	NS	<0.05	<0.05	<0.05

Mean values bearing the same superscript in a column did not differ significantly (P < 0.05)

[#]WHC= Water holding capacity ; [$]ERV=Extract release volume

455

456

Table 4. Effects of dietary inclusion of prebiotics on lipid oxidation parameter of fresh meat in broiler chickens (n = 15)

Group	Dietary treatments	TBARS* Value (mg MDA**/kg)		Free Fatty Acid (%)		Peroxide Value (meq/kg)	
		Breast	Thigh	Breast	Thigh	Breast	Thigh
T1	Control diet	0.21^{b}	0.16^{b}	0.006^{b}	0.008^{b}	1.42	1.29
T2	T1+ BMD@20 mg/kg	0.19^{ab}	0.14^{ab}	0.005^{ab}	0.006^{ab}	1.42	1.30
T3	T1+ 0.1% MOS	0.18^{ab}	0.13^{ab}	0.004^{ab}	0.006^{ab}	1.31	1.28
T4	T1+ 0.2% MOS	0.16^{a}	0.11^{a}	0.003^{a}	0.004^{a}	1.32	1.26
T5	T1+ 0.1% FOS	0.18^{ab}	0.14^{ab}	0.005^{ab}	0.007^{b}	1.32	1.27
T6	T1+ 0.1% FOS	0.19^{ab}	0.13^{ab}	0.004^{ab}	0.006^{ab}	1.30	1.25
SEM	-	0.02	0.03	0.002	0.03	0.39	0.57
P-value	-	<0.05	<0.05	<0.05	<0.05	NS	NS

Mean values bearing the same superscript in a column did not differ significantly ($P < 0.05$).

*TBRS=2-Thiobarbituric acid reacting substances; MDA** = Malondialdehyde

457

458

459

460

461

462

463

464

Table 5. Effects of dietary supplementation of prebiotics on anti-oxidant parameters of fresh meat in broiler chickens (n = 15)

Group	Dietary treatments	*ABTS+ (% inhibition)		**DPPH (% inhibition)	
		Breast	Thigh	Breast	Thigh
T1	Control diet	87.24	79.77	20.55[a]	13.96[a]
T2	T1+ BMD@20 mg/kg	86.38	69.05	20.43[a]	13.24[a]
T3	T1+ 0.1% MOS	87.95	89.05	22.54[ab]	14.78[ab]
T4	T1+ 0.2% MOS	87.38	79.81	24.33[b]	16.69[b]
T5	T1+ 0.1% FOS	87.19	69.57	22.18[ab]	14.50[ab]
T6	T1+ 0.1% FOS	86. 67	69.05	22.32[ab]	14.20[ab]
SEM	-	6.26	5.43	3.89	2.11
P-value		NS	NS	<0.05	<0.05

Mean values bearing the same superscript in a column did not differ significantly (P < 0.05).

ABTS + =2,2azino-bis -3-ethyl benzothiazoline-6-sulfonic acid; DPPH =2, 2-diphenyl-1-picrylhydrazyl

465

466

467

468

469

Table 6. Effects of dietary inclusion of prebiotics on microbiological load of fresh and refrigerated (14 d) meat in broiler chickens (n = 15)

Group	Dietary treatments	Microbial load (0d) *($log_{10}cfu/g$)*			Microbial load (14d) *($log_{10}cfu/g$)*		
		SPC*	Coli*	Stph*	SPC*	Coli*	Stph*
T1	Control diet	2.77^{b}	1.85^{b}	2.02^{b}	3.61^{b}	2.36^{b}	2.11^{b}
T2	T1+ BMD@20 mg/kg	2.50^{b}	1.25^{ab}	1.32^{ab}	3.28^{ab}	2.18^{ab}	1.64^{b}
T3	T1+ 0.1% MOS	2.01^{a}	1.05^{a}	1.19^{a}	3.02^{a}	1.99^{a}	1.33^{a}
T4	T1+ 0.2% MOS	2.32^{a}	1.11^{a}	1.24^{a}	3.05^{a}	2.06^{a}	1.34^{a}
T5	T1+ 0.1% FOS	2.36^{ab}	1.10^{a}	1.29^{ab}	3.15^{ab}	2.10^{a}	1.38^{a}
T6	T1+ 0.1% FOS	2.01a	1.10a	1.24a	3.32^{ab}	2.16^{ab}	1.40^{a}
SEM	-	0.03	0.04	0.09	0.06	0.03	0.05
P-value	-	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Mean values bearing the same superscript in a column did not differ significantly ($P < 0.05$).

*SPC= Standard plate count; *Coli= Coliform; *Stph= Staphylococcus aureus