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Influence of Inuloprebiotic Supplementation of the Diets of Broiler Chickens on Shelf-Life and Quality Characteristics of Meat

Park Sang-Oh and Park Byung-Sung
Department of Animal Biotechnology, College of Animal Life Science,
Kangwon National University, Chuncheon, Gangwon-Do, 200-701, Republic of Korea

Abstract: In the present study, as an alternative to antibiotics in broiler diets, microencapsulated-inuloprebiotics extracted from Korean Jerusalem artichoke was manufactured as a natural antimicrobial agents and added to broiler diets after which the quality and storability of chicken meat were investigated. A total of 360, 1 day old Ross 308 broiler chicks were randomly assigned to 3 treatment groups that were each replicated 4 times. The broilers were then divided into T1 (control), T2 (Avilamycin 8 g ton^{-1}) and T3 (inuloprebiotics 250 g ton^{-1}). The pH, water holding capacity and water content of chicken meat was significantly higher in T3 than T1 and T2. Additionally, the L^* value (lightness) and b^* value (yellowness) of the meat was significantly higher in T3 than T1 and T2. The TBARS value during low temperature storage of chicken thighs was significantly lower in T3 than T1 and T2. Finally, the sensory evaluation scores of the cooked chicken meat were significantly higher in T3 than T1 and T2. The results of this study suggest that the addition of inuloprebiotics as an alternative for antibiotics to broiler diets can greatly improve the quality and the storability of chicken meat.

Key words: Inuloprebiotics, water holding capacity, TBARS, sensory evaluations, chicken meat, Korea

INTRODUCTION

The appearance of super bacteria in livestock products has threatened the diets of humans therefore, production of safe animal foods without antibiotics is necessary. One such approach to this problem was to use inulin extracted from the Korean Jerusalem artichoke (*Helianthus tuberosus*) as a prebiotic for the promotion of antimicrobial effects in lieu of antibiotics (Rehman *et al*., 2008). Prebiotics are nondigestible food ingredients that are not broken down by the animal's upper gastrointestinal enzymes but are instead transferred to the large intestine where they stimulate the growth of bifidobacteria and lactobacilli among intestinal microorganisms, benefiting the host animals (Gibson and Roberfroid, 1995; Falaki *et al*., 2011). Since prebiotics have bifidogenic effects they are known as antimicrobial agents and immunomodulators that protect young animals from diarrhea and disease (Gibson and Rastall, 2006).

Inulin which is a linear fructan composed of a polymer of fructose molecules joined by a β (2→1) glycosidic bond is reportedly a prebiotic that is not hydrolyzed by gastric juices and digestive enzymes. As a result, >80% of ingested inuulin is transferred to the caecum and colon where it acts as an intestinal microorganism fermentation substrate with a bifidogenic effect that inhibits the growth of harmful intestinal strains while selectively stimulating the growth of beneficial bifidobacteria (Garcia *et al*., 2006; Rehman *et al*., 2008). FOS (Fructooligosaccharide; Xu *et al*., 2002), IMO (isomaltooligosaccharides; Zhang *et al*., 2003) and plant extracts (herb oil; Hernandez *et al*., 2004) which all have an antimicrobial growth promotion effect have been evaluated as prebiotics for poultry diets.

Ultimately, the addition of prebiotics to animal diets is expected to lead to intestinal microorganisms being balanced, leading to high productivity without antibiotics. However, when inulin is mixed directly with poultry diets as prebiotics, degeneration easily occurs during open-air distribution and storage. Therefore, when inulin that is directly mixed in the assorted diets is fed to animals, its prebiotic function is weakened because of weakened acid resistance in the stomach and small intestine and increased degradation absorption before inulin is transferred to the caecum and colon (Lopez-Molina *et al*., 2005; Park, 2008). To address this problem and maximize the prebiotic function, inulin must be homogenized by high pressure with Vitamin E, an antioxidant to manufacture microencapsulated-inuloprebiotics, a coating material soluble in the large intestine (Park, 2008;

Corresponding Author: Park, Department of Animal Biotechnology, College of Animal Life Science,
Kangwon National University, Chuncheon, Gangwon-do, 200-701, Republic of Korea

Son *et al*., 2008). Park (2008) reported that culturing inulin extracted from Korean Jerusalem artichoke *in vitro* according to previous studies resulted in the growth rate of beneficial intestinal *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Lactobacillus casei* strains being high in the inulin addition group while multiplication of harmful *Streptococcus aureus* and *Clostridium perfringens* strains was inhibited. Furthermore, selective multiplication of bifidobacteria and an increase in the thymic index and IgG were observed in the caecum of broilers fed microencapsulated-inuloprebiotics manufactured using inulin. Moreover, with the addition of inuloprebiotics to the broiler diets, the broiler productivity improved greatly when compared to the antibiotics addition group (Park and Park, 2009). However, almost no studies have been conducted to evaluate the quality characteristics and storability of safe chicken meat without antibiotics fed inuloprebiotics.

This study was carried out to investigate the quality characteristics and storability of the meat of chickens fed dietary microencapsulated-inuloprebiotics which prepared using inulin extracted from Korean Jerusalem artichoke.

MATERIALS AND METHODS

Inulin with an average DP (Degree of Polymerization) of 26 was extracted from Jerusalem artichoke using hot water as suggested by French (1989), after which it was freeze-dried. Next, inulin and water were mixed at a ratio of 1:1.2 (w/w) along with an antioxidant, vitamin E (α-tocopheryl acetate, 15 g ton^{-1}). The mixture was then homogenized using a high pressure homogenizer (T25 Basic, IKA, German) at 3,000×rpm while being heated at 55°C. An intestinal coating material, Sureteric (Colorcon, UK) which is strongly acidic to high pressure homogenate, stable in the stomach and the small intestine and soluble in the large intestine, was treated with compressed air (6 kgf cm^{-2}) to produce an ultrafine powder coating (high pressure homogenate 100: sureteric 2). The product was then dried using a spray dryer (B-191, Buchi, Sweiss) and manufactured as a microencapsulated-inuloprebiotic for a broiler feed additive. Particles of microencapsulated-inuloprebiotics were 150 μm and contained 15 g ton^{-1} of vitamin E and >96% inulin (Park, 2008; Son *et al*., 2008).

All experimental procedures including animals were conducted according to the scientific and ethical aspects reviewed by Swanson (2008). Experimental approval was obtained from the Institutional Animal Care and Use Committee (IACUC, Kangwon National University, South Korea). A total of 360, 1 day old Ross 308 male broiler chicks were randomly assigned to 3 treatment groups and there were 4 replicates of each treatment (30 birds per replicates). Specifically, animals were assigned to one of the following: T1 (control), T2 (Avilamycin 8 g ton^{-1}) or T3 (inuloprebiotics 250 g ton^{-1}). The level of inuloprebiotics added was determined based on data obtained from previous studies (Park and Park, 2009).

Table 1: Composition of experimental basal diets for broiler chickens (% as fed)

	Experimental basal diets	
Ingredients	Starter (0-21 days)	Grower (22-35 days)
Yellow corn ground	52.00	50.00
Soybean meal 44%	34.00	25.00
Corn gluten meal	4.70	5.70
Wheat meal	-	10.00
Soybean oil	5.00	5.00
Limestone	1.25	1.25
DCP	1.70	1.70
Salt	0.25	0.25
DL-Methionine	0.30	0.30
Lysine	0.30	0.30
Mineral premix[1]	0.34	0.34
Vitamin premix[2]	0.16	0.16
Total	100.00	100.00
Calculated values[3]		
ME (kcal kg^{-1})	3,100.00	3,150.00
Cp (%)	22.00	20.00
Lysine (%)	1.32	1.15
Methionine (%)	0.52	0.50
Methionine and cystine (%)	0.78	0.73
Calcium (%)	1.00	0.90
Available phosphorous (%)	0.45	0.40

[1]Supplied per kg of diet: iron ($FeSO_4.7H_2O$), 80 mg; zinc (ZnO), 80 mg; manganese, ($MnSO_4.H_2O$) 70 mg; copper ($CuSO_4.5H_2O$), 7 mg; iodine (Iodized NaCl), 1.20 mg; selenium (Na_2SeO_3), 0.30 mg; cobalt ($CoCl_2$), 0.70 mg. [2]Supplied per kg of diet: vitamin A (retinyl acetate), 10,500 IU; cholecalciferol, 4,100 IU; vitamin E (dl-α-tocopheryl acetate), 45 mg; menadione, 3.0 mg; thiamine, 2.5 mg; riboflavin, 5 mg; pyridoxine, 5 mg; choline, 150 mg; cobalamin, 0.02 mg; biotin, 0.18 mg; niacin, 44 mg; pantothenic acid, 17 mg; folic acid, 1.5 mg. [3]Calculated values from National Research Council (1994)

Corn and soybean meal were added to the experimental diet in quantities sufficient to satisfy or exceed the nutrient requirements for broilers suggested by the National Research Council (1994) feeding standards and the levels of antibiotics and inuloprebiotics added were adjusted by reducing the amount of corn. The crude protein and metabolic energy content were adjusted to similar levels (Table 1). The assorted experimental diet was stored in a cool place and provided to animals with water *ad libitum*. For 35 days after hatching, broilers were bred under standard conditions (density 10 broiler chicks m^{-2}) and each pen was underlain with 10 cm of chaffs for bedding. Broilers were bred differently in the first half period (0-21 days) and the second half period (22-35 days) with the temperature of the breeding room being maintained at 33°C from the 1st day to the 3rd day and then lowered by 2-3°C $week^{-1}$ afterwards until 25°C was reached on the 22nd day where it was maintained for the

duration of the study. The room was maintained at 70% humidity and 24 h continuous illumination and was ventilated 3-5 times day^{-1} an automatic ventilation system.

After the breeding experiment was complete, 40 randomly selected broilers (10 having a near average body weight from each replicate) were sacrificed using CO_2 according to the test animal euthanasia recommendations (Close *et al*., 1997). To minimize the stress on broilers, selected broilers were separated from the rest of the animals 1-2 h before sacrificing. They were then exposed to 100% CO_2 in an acrylic euthanasia box. The carcasses were then immersed in hot water (58-60°C) for 4 min after which they were passed through the defeathering equipment for 2 min. Evisceration was conducted 15 min after sacrifice and the carcasses were kept at 18°C for 1 h after death after which they were stored at 4°C in the chilling room for up to 24 h after death. For testing materials, pH was measured in the half section of the carcass of 40 broilers per treatment group on the day of sacrifice. Chicken breasts for the analysis of water holding capacity and meat color and chicken thighs for the measurement of TBARS were sampled from 28 carcasses per treatment group 24 h after death and the sensory evaluation was conducted for 12 carcasses per treatment group.

Using a portable pH meter (Crison 507, Crison, Milan, Italy) equipped with an insertion glass electrode calibrated with pH 4.0 and 7.00 buffer solutions at ambient temperature, the pH of the chicken meat was measured in the right breast of the carcass before deboning within 3 h of euthanasia by placing the insertion glass electrode directly on the meat (Berri *et al*., 2008).

To measure the water holding capacity (WHC), 10 g of breast was kept in a 70°C water bath for 30 min and then preserved at 4°C for 48 h before drip loss was measured through centrifugation at 3,500×rpm for 10 min. In addition, the water content was measured to determine the water holding capacity (%) as follows: water content-drip loss/water content×100 (Honikel, 1998).

The chicken meat color was determined using a Chroma meter (CR-300, Minolta, Osaka, Japan). After removing the surface of the muscle, the color was developed from the samples at 4°C for 1 h prior to measurement. All measurements were repeated four times using white tile ($L^* = 92.30$, $a^* = 0.32$ and $b^* = 0.33$) as a standard. Meat color was described based on the Hunter value (L^* = lightness, a^* = redness and b^* = yellowness).

The TBARS (Thiobarbituric Acid Reactive Substances) value of the chicken meat was measured as described above. Specifically, 10 g of thigh muscle with skin that had been stored in a 4°C chilling room for 24 h after death was placed in a polypropylene plastic bag and then heated in a 70°C water bath for 10 min so that lipid oxidation occurred to hydrolyze all Malondialdehyde Acids (MDA) bonded with the meat protein after which the sample was placed in an oxygen permeable polyethylene ziplock bag and stored at 4°C for 7 day. The amount of TBARS reactant was measured according to the method described by Buege and Aust (1978) to determine the degree of lipid oxidation based on analysis of the secondary metabolite, MDA. Briefly, 5 g of chicken thigh and 15 mL of distilled water were mixed and homogenized at 3,500×rpm for 5 min using a homogenizer (Tissue grinder 1102-1, Japan). Next, 1 mL of homogenate, 50 μL (72%) of butylated hydroxyanisol and 2 mL of 50% trichloroacetic acid mixture (TBA/TCA) containing 1.3% (wt/vol) thiobarbituric acid dissolved at 60°C were added and mixed. For color development, the mixture was heated in a 60°C water bath for 1 h and then frozen, after which it was centrifuged at 3,500×rpm for 15 min to obtain a supernatant. The absorbance at 531 nm was measured from the supernatant using a spectrophotometer (UV mini-1240, Shimadzu, Japan) after which it was compared with the measured value of the blank containing 1 mL of distilled water and 2 mL of TBA/TCA mixture and the TBARS value in the chicken meat was calculated based on its difference multiplied by the common coefficient 5.88 and expressed in MDA (malondialdehyde) g ton^{-1}. For MDA formation, tetrathoxypropane (Sigma, St. Louis, MO) self-degrading in a water solution was used as a standard.

Sensory evaluation of taste was conducted in a sensory evaluation room for all of the chicken meat except that from the antibiotics addition group within 30 min of being fully cooked in boiled water. For the taste panel, 14 college students (7 males and females) were selected and trained. The evaluation items were taste and flavor, meat color, juiceness, texture and overall acceptability of chicken meat for which a 9-point scale in which higher numbers indicated greater acceptability was used.

Statistical analysis: All chemical determinations were carried out 4 time. Treatment means of data were analyzed using the one-way ANOVA procedure of SAS (1998) for analysis of variance. Significant differences among treatment groups were determined at 5% level ($p<0.05$) by Duncan's multiple range tests.

RESULTS AND DISCUSSION

The pH, water holding capacity and moisture content of the chicken meat are shown in Table 2. The pH of T3 was significantly lower than that T1 and T2 while the

Table 2: Effect of dietary inuloprebiotics on pH, moisture in whole chicken meats and WHC in chicken breast muscle[1]

Item	T1	T2	T3	SEM[3]	p-value
pH	6.51[a]	6.52[a]	6.22[b]	0.0366	0.012
WHC[2] (%)	55.66[b]	55.82[b]	57.80[a]	0.3240	0.030
Moisture (%)	74.88[b]	75.05[b]	75.85[a]	0.0323	0.027

[1]T1: control, T2: avilamycin 8 g ton^{-1}, T3: inuloprebiotics 250 g ton^{-1}. [2]WHC: Water Holding Capacity. [3]SEM: Standard Error of the Mean values. [a,b]Mean values with different superscripts are significantly different at p<0.05

Table 3: Effect of dietary inuloprebiotics on TBARS values in chicken thigh muscle with skin during storage days at 5°C[1]

Storage days	T1	T2	T3	SEM[2]	p-value
0	0.17	0.17	0.17	0.0035	0.002
3	0.32[a]	0.36[a]	0.20[b]	0.0248	0.001
5	0.60[a]	0.59[a]	0.49[b]	0.0218	0.030
7	0.91[a]	0.82[a]	0.65[b]	0.0438	0.015

[1]T1: control, T2: avilamycin 8 g ton^{-1}, T3: inuloprebiotics 250 g ton^{-1}; TBARS: Thiobarbituric Reactive Substance (malondialdehyde g ton^{-1} of meat). [2]SEM: Standard Error of Mean values. [a,b]Mean values with different superscripts are significantly different at p<0.05

water holding capacity and moisture content of T3 was significantly higher than that of T1 and T2. Conversely, the pH, water holding capacity and moisture content did not differ significantly between T1 and T2.

The drip loss or water loss rate for the water holding capacity measurement has been broadly studied with respect to its effect on nutrient content in muscle, flavor, color, hardness and taste. The pH and water holding capacity of chicken meat greatly affect the quality of chicken meat with water holding capacity influencing the economic efficiency of the processing industry and consumer preference (Tian and Yu, 2001). Chicken meat with low pH is assumed to be related to the method of sacrifice and time of measurement after sacrifice (Van Laack *et al*., 2000; Young *et al*., 2003). Additionally, low pH and high water holding capacity were found in an inuloprebiotics intake group among the broilers sacrificed by CO_2 euthanasis with minimum stress (Qiao *et al*., 2001; Young *et al*., 2003). The increase in water holding capacity in the inuloprebiotics addition group was attributed to the improved antioxidative state in response to the lowered pH of the carcass after death and the high water content and TBARS inhibition (Table 3) (Nissen and Young, 2006). However, the reason for the low pH and high water holding capacity in chicken meat has not yet been elucidated therefore, further studies in this area necessary. Qiao *et al*. (2001) reported that a treatment group with low pH and high water content in ground chicken meat with different meat colors had a higher water holding capacity than a treatment group with high pH and low water content. Young *et al*. (2003) reported that the pH of broilers sacrificed calmly with minimum stress showed no significant difference at 30 min after death and 4 h after death while the pH of broilers sacrificed in the slaughterhouse under high levels of stress differed significantly at every hour after death and unlike previous studies, decreased pH did not decrease the water holding capacity.

Table 4: Effect of inuloprebiotics on meat color in breast muscle from broiler chickens[1]

Item[2]	T1	T2	T3	SEM[3]	p-value
L*	51.59[b]	51.88[b]	55.18[a]	0.6128	0.002
a*	2.64[a]	2.20[b]	2.52[a]	0.0722	0.005
b*	3.20[b]	3.11[b]	3.44[a]	0.0577	0.015

[1]T1: control, T2: avilamycin 8 g ton^{-1}, T3: inuloprebiotics 250 g ton^{-1}. [2]L* (lightness), a* (redness), b*(yellowness). [3]SEM: Standard Error of the Mean values. [a,b]Mean values with different superscripts are significantly different at p<0.05

Table 5: Effect of dietary inuloprebiotics on sensory evaluation of boiled chicken breast meat

Treatments[1]	Taste and flavor	Color	Juiciness	Texture	Total acceptability
T1	7.8500[b]	7.6500[b]	7.8700[b]	7.8200[b]	7.8700[b]
T2	7.7000[b]	7.8000[b]	7.9100[b]	7.7700[b]	7.8000[b]
T3	8.8800[a]	8.7900[a]	8.3900[a]	8.7400[a]	8.5300[a]
SEM[2]	0.3171	0.1810	0.1763	0.3353	0.1829
p-value	0.0030	0.0020	0.0300	0.0010	0.0020

[1]T1: control, T2: avilamycin 8 g ton^{-1}, T3: inuloprebiotics 250 g ton^{-1}. [2]SEM: Standard Error of Mean values. [a,b]Mean values with different superscripts are significantly different at p<0.05

pH is one of the most important physical variables in quality evaluation of meat quality and is therefore, widely used as a prediction index of the technological and sensory quality of the meat (Young *et al*., 2004). It has been reported that muscle pH directly reflects the levels of lactic acid and glycogen in muscle and affects shear force, drip loss and meat color (Briskey and Pedersen, 1961; Young *et al*., 2003). Generally, since the pH of chicken meat sacrificed under stress in the slaughterhouse can change during rigor mortis, the last pH measured in the 24th h after completion of rigor mortis is used for quality evaluation of chicken meat. It is well known that if the last pH of the meat is low, the water holding capacity decreases and the appearance of paleness and low water holding capacity are related to or caused by low pH (Van Laack *et al*., 2000). If water holding capacity decreases in muscle, meat juice can flow out and soluble nutrients and flavor can be lost causing muscle to become dried and hardened and taste to be lost, consequently lowering the meat quality (Tian and Yu, 2001).

The meat color of chicken breast is shown in Table 4. The L* value (representing lightness) and the b* value (representing yellowness) of the meat colors was significantly higher for T3 than T1 and T2 while T1 and T2 did not differ significantly. Based on the results of this study, the higher lightness and yellowness of the inuloprebiotics addition group was attributed to low pH, high water holding capacity (Table 2) and low TBARS values (Table 3). The increase in the water holding

capacity caused by the increased antioxidative state in chickens is related to temperature, pH and meat color expression following death (Young *et al*., 2003; Nissen and Young, 2006). Chicken meat color is a critical standard affecting consumer's evaluation of meat quality and the L^* value is important in white muscle and related to drip loss and pH (Barbut, 1997). Young *et al*. (2003) found that meat color of chicken muscle tissue showed a higher lightness value than chicken meat when the pH was lower which is consistent with the results of the present study.

The change in the TBARS value during low temperature storage of chicken thighs with skin is shown in Table 3. The TBARS value increased significantly as the number of storage days increased. After the 3rd day, the TBARS value of T3 was significantly lower than that T1 and T2 while there was no significant difference between T1 and T2. A novel finding of this study is that improved storability of chicken meat can be expected in response to the addition of inuloprebiotics to the diets of broilers. The fact that the addition of inuloprebiotics induced a low TBARS value can be attributed to the effects of antioxidative and antimicrobial activities of inuloprebiotics (Park and Park, 2009). Park (2008) reported that the addition levels of inuloprebiotics stimulated the growth of beneficial microorganisms in the caecum of broilers which promoted immune response and antioxidative activity of vitamin E, ultimately leading to improved broiler productivity. Moreover, their report suggested that inulin is highly effective at inhibiting the growth of harmful *in vitro* bacteria and stimulating the growth of beneficial microorganisms (Park, 2008). Lipid oxidation leads to reduced meat quality and malondialdehyde is a soluble hydrolysate of lipids widely used as an index for reflecting lipid oxidation in meat (Raharjo and Sofos, 1993). It has been reported that the addition of herbs extracted from plants to animal feed helps the health of animals with various chemical materials in herbs and if animals are fed these herbs, the chemical materials or phytochemical extracts will promote a self-protecting ability against insects, mold, bacteria and viruses and had longtime contact between plant extracts and carcasses promotes the antimicrobial effect of chemical materials (Dickens *et al*., 2000). Vitamin E provided through diets supplemented with inuloprebiotics has been found to induce a residual effect or reproduction of tocopherol in diets, inhibiting TBARS production in chicken meat (Young *et al*., 2003). Since the increased antioxidative state possesses a strong protective capability for the stress caused by lipid oxidation, the inhibitory effect of TBARS in response to the addition of inuloprebiotics is considered to indicate improved storability of chicken meat.

The sensory evaluation scores of cooked chicken meat are shown in Table 5. The scores of taste and flavor, color, juiciness, texture and total acceptability of chicken meat were significantly higher in T3 than T1 and T2. Overall, the results of this study indicate that the addition of inuloprebiotics to broiler diets might improve the taste of chicken meat. The high sensory evaluation scores in the inuloprebiotics addition group are related to the high water content, water holding capacity (Table 2) and meat color (Table 4) as well as the low TBARS value in chicken meat (Table 3) (Tian and Yu, 2001).

CONCLUSION

On the basis of the results, it can be concluded that the utilization of inuloprebiotics as an alternative for antibiotics to broiler diets improved the shelf-life, acceptability and quality of meat.

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