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Effect of Dietary Microencapsulated-Inulin on Carcass Characteristics and Growth Performance in Broiler Chickens

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Abstract: In the present study, we investigated the effect of Microencapsulated-Inulin (MCI) in feed on the improvement of the growth performance in broilers using MCI prepared from Korean Jerusalem artichoke as a natural antibacterial growth promoter. After sex identification, 320 male Ross 308 broilers were randomly allotted to treatment groups and fed for 35 days. Treatment groups consisted of T1 (no supplementation; control), T2 (avilamycin, 8 g ton^{-1}), T3 (MCI, 200 g ton^{-1}) and T4 (MCI, 250 g ton^{-1}). The growth performance and the dressing percentage were higher in broilers in groups T3 and T4 than in broilers in groups T1 and T2; statistical significance in the differences among the treatment groups was verified. The weights of breast and thigh muscles were significantly higher in broilers in T3 and T4 than in T1 and T2 and abdominal fat was significantly lower in broilers in T3 and T4 than in T1 and T2 with a decrease of 19.08-23.30%. The levels of blood immunoglobulins, IgG, IgM, IgA and weights of thymus and bursa of fabricius were significantly greater in T3 and T4 compared with T1 and T2 with an increase of IgG, IgM and IgA being 125.1-168.5, 100.5-170.5 and 103.0-125.3%, respectively. The colony counts of the beneficial intestinal microorganisms, Bifidobacteria and Lactobacillus were significantly greater in T3 and T4 than in T1 and T2 but the counts of harmful *E. coli* and Salmonella were significantly less in T3 and T4 than in T1 and T2. The supplementation of broiler feed with 200 g ton^{-1} microencapsulated-inulin can significantly improve the productivity of broiler chickens.

Key words: Microencapsulated-inulin, performance, carcass, immunoglobulin, microflora, Korea

INTRODUCTION

The appearance and increased prevalence of antibiotic-resistant bacteria has become a serious social and medical issue and has spurred the abolition of antibiotic supplementation of livestock feed and the adoption of more eco-friendly organic livestock farming. Discontinuing the use of antibiotics in livestock feeds in factory-style livestock farming can diminish the productivity of livestock animals, necessitating the development of antibacterial growth promoters from natural substances (Dibner and Richards, 2005; Shakibaie *et al*., 2009).

Prebiotics are non-degradable dietary ingredients that reach the large intestine without being hydrolyzed in the upper alimentary tract. Prebiotics benefit the host animal by stimulating the selective growth and/or activity of microorganisms, Bifidobacteria in particular and repressing microbial growth in the large intestine (Gibson and Rastall, 2006; Falaki *et al*., 2011). The growth suppression and immunomodulatory (i.e., bifidogenic) effects enable prebiotics to preventing diarrhea in young animals and promote the growth of livestock animals (Patterson and Burkholder, 2003).

Inulin (Rada *et al*., 2001), fructooligosaccharide (Xu *et al*., 2002) isomalto-oligosaccharides (IMOs) (Zhang *et al*., 2003) and essential oil (Hernandez *et al*., 2004) are representative prebiotics. Especially, inulin is a linear fructose polymer connected by β (2→1) glycosidic bonds. Inulin is not degraded by gastric juice and digestive enzymes in animals and >80% of it reaches the largest intestine and is available to microorganisms as a fermentation substrate. The fermentation activity inhibits the growth of harmful strains, selectively stimulates the growth of beneficial bifidobacteria and thus promotes the growth of broiler chickens (Lopez-Molina *et al*., 2005; Rehman *et al*., 2008; Rebole *et al*., 2010). However, inulin is also vulnerable to degeneration by air during commercial distribution and has a low transit rate from the upper alimentary tract to the caecum. To address these problems, the production of Microencapsulated-Inulin (MCI) using Sureteric has been developed (Park, 2008; Son *et al*., 2008).

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A previous *in vitro* investigation of the antibacterial activity of inulin from Korean Jerusalem artichoke (*Cynara scolymus* L.) reported that compared with the control group broiler chickens consuming an inulin-supplemented diet displayed higher growth rates of the beneficial bacteria *Bifidobacterium longum*, *B. bifidum*, *Lactobacillus acidophilus* and *L. casei* and limited growth of the harmful bacteria *Streptococcus aureus* and *Clostridium perfringens* (Park, 2008). The same study reported the observation of the selective proliferation of bifidobacteria as well as thymus index and immunoglobulin G in broiler chickens whose diet was supplemented with MCI. However, optimization of the MCI was beyond the scope of the study.

The present study produced MCI from Korean Jerusalem artichoke exactly as described previously and investigated in more depth its effect on the productivity of broilers and as an antibacterial growth promoter when supplied in feed.

MATERIALS AND METHODS

Inulin with a mean degree of polymerization of 26 was extracted from Korean Jerusalem artichoke using the hot water and cooling method proposed by French (1989). Briefly, the extract was prepared in powder form after freeze-drying. Inulin and vitamin E (α-tocopheryl acetate, 15 g ton^{-1}) as an antioxidant was added and mixed with warm (70°C) water at an inulin: vitamin E ratio of 1:1.2 (w/w). A T25 Basic high pressure homogenizer (IKA, Wilmington, NC, USA), the mixture was pressure homogenized and then mixed with Sureteric (Colorcon, Dartford Kent, UK) which is stable in the strongly acidic stomach and small intestine but dissolves in the large intestine at a 9:1 w/w ratio of 9:1 homogenate: Sureteric. The final inulin: vitamin E preparation was encapsulated and added to the broiler feeds (Park, 2008; Son *et al*., 2008).

All scientific procedures including animal experiments were performed in accordance with the scientific and ethical aspects reviewed by Swanson (2008) and were approved by the Institutional Animal Care and Use Committees of Kangwon National University, South Korea. After sex identification, 320, 1 day old male Ross 308 broiler chicks were randomly allotted to four feed treatment groups with four replicate 20 broiler pens per treatment group: T1 (unsupplemented feed; control), T2 (feed supplemented with avilamycin of 8 g ton^{-1}), T3 (feed supplemented with MCI of 200 g ton^{-1}) and T4 (feed supplemented with MCI of 250 g ton^{-1}). The amount of MCI was based on the results of the previous study (Park, 2008).

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

	Experimental basal diets	
Ingredient	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)

The experimental diets were prepared mainly with corn and soybean meal to satisfy or exceed the minimum requirement of nutrients for broilers specified in the NRC National Research Council (1994). The amounts of antibiotic (avilamycin) or MCI in the diets were adjusted by replacing the equal amount of corn. First, primary mixtures were prepared with 20-25 g of MCI in 1 kg of mixture and added to the basal diet to contain 0.10% of the mixture so that the concentration of the MCI was 200 and 250 g ton^{-1}, respectively. The content of crude protein and metabolic energy were adjusted at the equivalent levels (Table 1). The diets were stored in a cool place and fed *ad libitum* with water. Broilers were kept under standard condition (10 broiler chicks m^{-2}) for 35 day from hatching. Each pen was covered with a 10 cm thick layer of rice husks. Feeding period was divided into the starter period (days 0-21) and the grower period (days 22-35) and the temperature in the feeding room was kept at 33°C for the first 3 days and decreased by 2-3°C every week until being maintained at 25°C from day 22. The relative humidity was kept at 70% and illumination was constant. The air ventilation system was operated 3-5 times a day to supply fresh air.

The growth performance of broilers were assessed at each stage of growth by measuring feed intake, weight gain and feed conversion ratio at 3 and 5 weeks from

hatching. The feed conversion ratio was calculated by dividing the feed intake for a given period of time by the weight gain for the same period. At the end of all experiments, 16 broilers were selected from each treatment group (four broilers from each replicate pen) and killed by means of cervical dislocation in a stress-free manner in accordance with the recommended method of euthanasia for experimental animals (Close *et al*., 1997). The dressing percent was calculated as a ratio of carcass weight (total weight less feathers, blood, head, legs and intestines) against live weight. The head of each sacrificed bird was cut off at the first neck bone and the legs at the knees. The weight ratio of the leg muscles including the breast and skin was calculated as a ratio against carcass weight. The weights of liver, gizzard, abdominal fat and immune organs (thymus, spleen, bursa of Fabricius) were calculated as ratios against live weight. The weight of abdominal fat was measured as the weight of all fat around the abdominal cavity and gizzard.

At the end of the experiments, 12 broilers were selected randomly from each treatment group (three from each replicate pen) and 1 mL of blood was collected from a wing vein using a plain tube (Greine, Co Ltd., Australia). Serum was obtained from the blood using a Sorvall RC-3 centrifuge (Thermo Scientific, Pittsburgh, PA, USA) operating at 3,000×rpm for 15 min at 4°C. The separated serum was then frozen rapidly using liquid nitrogen and stored at -20°C until used for analysis. Serum immunoglobulin was quantified using an performed in enzyme-linked immunosorbent assay (ELISA; Bethyl Laboratories, Montgomery, TX, USA) proposed by Mockett and Rose (1986). After the reaction process using IgG (chicken IgG ELISA quantitation set, E30-104; Bethyl Laboratories), IgA (chicken IgA ELISA quantitation set, E30-103; Bethyl Laboratories) and IgM (chicken IgM ELISA quantitation set, E30-102; Bethyl Laboratories), the assay of antibody was performed by measuring the optical density at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

About 16 sacrificed broilers were selected from each treatment group (four from each replicate pen) and caeca were aseptically collected to investigate the intestinal microorganisms. The collected caeca were kept in anaerobic state in sealed anaerobic jars (Oxoid, Basingstoke, UK) with AnaeroGen sachets (Oxoid). The cecal content was subjected to a homogenization process and diluted 10 times using 9 mL of sterile phosphorus buffered saline (PBS, 0.1 M, pH 7.0) for each 1 g (1:9, w/v). The dilution process was continued using sterile anaerobic saline for the calculation of coefficients. All procedures were performed in anaerobically in an anaerobic chamber (5% hydrogen, 5% CO_2, balanced nitrogen). Microorganisms were cultured by streaking 100 μL of diluted sample (10^2-10^7) sample on a sterile plate medium. Different medium was used for each type of microorganism: *Lactobacillus* sp. (MRS agar; Oxoid); *Bifidobacterium* sp. (bifidobacterium selective agar; BIM-25 medium; Munoa and Pares, 1988); Salmonella (SS agar; Difco, Detroit, MI, USA); *Escherichia coli* (McConkey Purple agar; Difco, Detroit, MI, USA). *Salmonella* and *E. coli* were cultured in aerobic condition for 24 h at 37°C and *Lactobacillus* sp. and *Bifidobacterium* sp. were cultured in an anaerobic and static condition in sealed anaerobic jars with AnaeroGen sachets for 48 and 72 h, respectively at 37°C. The number of colonies was then counted to determine the colony forming unit (cfu) per gram of fresh cecal content which was then indicated in common logarithms.

Statistical analysis: All collected data was subjected to General Linear Models procedure for the analysis of variance using SAS (1998) software and verified for statistic significance ($p<0.05$) at 95% confidence level using Duncan's multiple range test.

RESULTS AND DISCUSSION

Growth performance: The growth performance of broilers fed with MCI as a dietary supplement for 35 day is shown in Table 2. The weight of broilers was significantly higher in T3 and T4 compared with T1 and T2 and in T2 compared with T1 during the entire experimental period (days 0-35) although, there was not a statistically significant difference between T2 and T3 during the starter period. Feed intake was significantly greater in T3 and T4 compared with T1 and T2 but there was no significant difference between T3 and T4. Broilers in group T2 consumed a significantly greater amount of feed compared with T1 birds. The feed conversion ratio for entire experimental period in T4 was the highest with statistically different from T3 and T4 while there was no significant different between T1 and T2 among T2, T3 and T4.

Notably, the effect of MCI on weight gain reached a plateau at a certain level without further increase when its concentration was 200 g ton^{-1} or higher. Therefore, 200 g ton^{-1} was considered to be the optimal concentration of MCI in the diets of broiler chickens.

The reason for the higher weight gain in the broilers who received MCI may have reflected the promotion of the growth of the beneficial *Lactobacillus* and *Bifidobacteria* bacteria in the caecum (Table 3) with health of the birds improved and feed intake stimulated as

Table 2: Growth performance of broilers fed the experimental diets for 35 days[1]

Days	T1	T2	T3	T4	SEM[2]	p-value
Body weight gain (g)						
0-21	795[c]	837[b]	861[a]	860[a]	6.9785	0.001
22-35	1,008[c]	1,068[b]	1,143[a]	1,140[a]	8.9515	0.003
0-35	1,813[d]	1,905[c]	2,004[b]	2,000[a]	21.0584	0.0001
Feed intake (g)						
0-21	1,302[c]	1,347[b]	1,385[a]	1,384[a]	10.1280	0.0001
22-35	1,637[c]	1,716[b]	1,772[a]	1,778[a]	16.1753	0.0001
0-35	2,939[c]	3,063[b]	3,157[a]	3,162[a]	25.8917	0.0001
Feed conversion ratio[3]						
0-21	1.63[a]	1.60[b]	1.60[b]	1.60[b]	0.0047	0.0001
22-35	1.62[a]	1.60[a]	1.55[b]	1.56[b]	0.0126	0.0001
0-35	1.62[a]	1.60[ab]	1.57[bc]	1.58[b]	0.0068	0.0001

[1]T1: control, T2: avilamycin 8 g ton^{-1}, T3: MCI 200 g ton^{-1}, T4: MCI 250 g ton^{-1}. [2]Standard error of the mean values. [3]Feed conversion ratio is feed intake/body weight gain. [a-d]Mean values with different superscripts differ significantly ($p<0.05$)

Table 3: Viable cell counts of microflora in cecal digesta of broilers fed the experimental diets for 35 days[1] (log 10 cfu g^{-1} content)

ItemS	T1	T2	T3	T4	SEM[2]	p-value
Bifidobacteria	6.07[d]	7.21[c]	8.53[b]	8.63[a]	0.2599	0.0001
Lactobacillus	6.81[c]	7.77[b]	8.55[a]	8.92[a]	0.2115	0.0001
E. coli	8.95[a]	7.84[b]	6.36[c]	6.65[c]	0.2491	0.0001
Salmonella	8.78[a]	7.63[b]	6.30[c]	6.34[c]	0.3167	0.0001

[1]T1: control, T2: avilamycin 8 g ton^{-} , T3: MCI 200 g ton^{-} , T4: MCI 250 g ton^{-1}. [2] Standard error of the mean values. [a-d]Mean values with different superscripts differ significantly ($p<0.05$)

Table 4: Indices of main immune organs and immunogloblin of 35 days old broilers fed inuloprebiotics-supplemented diets[1]

Parameters[2]	T1	T2	T3	T4	SEM[3]	p-value
IgG (μg mL^{-1})	65.51[d]	79.67[c]	96.54[b]	103.67[a]	4.4887	0.0001
IgM (μg mL^{-1})	40.17[d]	63.27[c]	64.56[b]	65.38[a]	2.7369	0.0001
IgA (μg mL^{-1})	38.01[d]	43.28[c]	45.63[a]	44.14[b]	0.8314	0.0001
Thymus	0.09[c]	0.15[b]	0.20[a]	0.19[a]	0.0136	0.0001
Spleen	1.05[b]	1.49[a]	1.58[a]	1.59[a]	0.0582	0.0001
Bursa of fabricius	1.90[c]	2.40[b]	2.68[a]	2.80[a]	0.1094	0.0001

[1]T1: control, T2: avilamycin 8 g ton^{-1}, T3: MCI 200 g ton^{-1}, T4: MCI 250 g ton^{-1}. [2]Organ weight: g kg^{-1} live body weight. [3]Standard error of the mean values. [a-d]Mean values with different superscripts differ significantly ($p<0.05$)

immunoglobulins increased due to the increased weight gain of the immunological organs (thymus, spleen and bursa of Fabricius) (Table 4). Increased weight gain in birds animals that had improved health due to immunologic competence of prebiotics and its effect of increasing the active antibacterial substances have previously been reported (Cetein *et al*., 2005). The observations that the β (2→1) glycosidic bond of inulin is resistant to the actions of digestive enzymes in broilers, allowing the compound to promote the growth of beneficial microorganisms while inhibiting the growth of harmful ones (Rehman *et al*., 2008) agrees with the present results. The reported weight gain after feeding broilers with prebiotics such as FOS (Xu *et al*., 2003), IMO (Zhang *et al*., 2003), vegetable extract (Hernandez *et al*., 2004) or inulin (Rebole *et al*., 2010) also agree with the current results.

Table 5: Characteristics of carcass of broilers fed the experimental diets for 35 days[1]

Items[2]	T1	T2	T3	T4	SEM[3]	p-value
Carcass weight (g)	1,305[c]	1,403[b]	1,450[a]	1,465[a]	20.6254	0.0001
Dressing percentage	71.40[c]	72.56[b]	73.25[a]	73.18[a]	0.2077	0.0001
Breast muscle (%)	18.65[c]	19.24[b]	19.78[a]	19.80[a]	0.1653	0.0001
Thigh muscle (%)	16.57[c]	17.33[b]	18.27[a]	18.28[a]	0.2022	0.0001
Gizzard (%)	1.78	1.75	1.79	1.80	0.0271	0.904
Liver (%)	2.88	2.90	2.87	2.90	0.0220	0.502
Abdominal fat (%)	1.76[a]	1.73[a]	1.54[b]	1.43[c]	0.0587	0.001

[1]T1: control, T2: avilamycin 8 g ton^{-1}, T3: MCI 200 g ton^{-} ; T4: MCI 250 g ton^{-1}. [2]Carcass weight relative to live body weight; Breast and thigh muscle weight relative to carcass weight. Gizzard, liver and abdominal fat weight relative to live body weight. [3]Standard error of the mean values. [a-c]Mean values with different superscripts differ significantly ($p<0.05$)

Carcass characteristics: The characteristics of the carcasses of broilers treated with the MCI are summarized in Table 5. Carcass weight and dressing percentage were significantly higher in T3 and T4 compared with T1 and T2. While no significant difference could be observed between T3 and T4, results in T2 were significantly higher than in T1. The weights of breast and thigh muscle were higher in T3 and T4 than in T1 and T2 with a relatively higher result in T2 than in T1. There were statistically significant differences among treatment groups although, the difference between T3 and T4 was not significant. The weights of liver and gizzard showed no difference among the treatment groups but abdominal fat was significantly less in T3 and T4 compared with T1 and T2; there was no difference between T1 and T2.

One of the most notable findings of the study was that the abdominal fat in T3 and T4 was lower by 19.08 and 21.97%, respectively when compared with T1 and by 20.45 and 23.30%, respectively, when compared with T2. In lipid metabolism, blood lipids migrate to living tissues where they are used for the generation of energy with the remainder being stored in abdominal tissues. The fact that abdominal fat decreased in this study is probably because the amount of lipid that moved to the abdominal tissues decreased due to the lowering of blood lipid by dietary inulin (Velasco *et al*., 2010). As inulin was known to reduce the blood lipid level in human and animal studies, the low abdominal fat level in broiler fed a diet supplemented with MCI in the present study can be considered the result of inulin-mediated lipid reduction in biological tissues (Tokunaga *et al*., 1986; Fiordaliso *et al*., 1995).

Kok *et al*. (1996) reported that triglyceride-very low density lipoprotein in blood in rats treated with 10% oligofructose decreased significantly and Davidson *et al*. (1998) reported that total cholesterol and low density lipoprotein cholesterol levels in blood in high blood cholesterol patients were reduced significantly after treatment with inulin-containing chichory. Both studies support the present results.

Immunoglobulins: Change of the weight of serum immunoglobulins and immunologic organs in broilers fed with MCI are shown in Table 4. The serum concentration of IgG, IgM and IgA increased significantly in T3 and T4 compared with T1 and T2. Values of immunoglobulin were significantly higher in T4 than in T3 and in T2 than in T1. The increase of serum IgG was higher in T3 and T4 by 155.6 and 168.5%, respectively when compared with T1 and by 125.1 and 135.5%, respectively when compared with T2. IgM level was higher in T3 and T4 by 167.8 and 170.5%, respectively when compared with T1 and by 100.5 and 102.2%, respectively when compared with T2. The IgA level was higher in T3 and T4 by 125.3 and 105.9%, respectively when compared with T1 and by 121.9 and 103.0%, respectively when compared with T2.

A higher serum IgG level in broilers treated with MCI is evidence that MCI are effective in improving humoral immunity (Park, 2008).

Immunoglobulins are produced in B-cells in bone marrow and the biological characteristics of IgG, IgA and IgM in poultry are similar to those of immunoglobulins in mammals. Since IgG is present at the highest concentration and is responsible for immunologic competence, the immunopotency of serum IgG can be used as an index of humoral immunity (Higgins, 1975). The increased serum IgG in Turkeys treated with MOS (Savage *et al*., 1996; Cetein *et al*., 2005) support the present studies.

The thymus and the bursa of Fabricius in broilers measured as the percentage of live weight were significantly greater in T3 and T4 compared with T1 and T2. While there was no statistically significant difference between T3 and T4, measurements in T2 were significantly higher than in T1. Weights of spleen in T2, T3 and T4 were similar among the 3 groups but all three treatment groups showed significantly higher results than in T1.

The thymus is an important organ for the production of antibodies. The thymus data in this study suggests that MCI increased the proliferation capability of spleen cells. The immune system of broilers is different from that of mammals such as rats and mice. The weight of the bursa of Fabricius is relatively consistent in poultry. The bursa of Fabricius has been used in the studies of the development of B-lymphocytes and functional development. As broilers grow, their thymus and bursa of Fabricius develop and the immunological reaction becomes dependent on the spleen and lymph nodes. Controlling the immune status of broilers may have a beneficial effect on the health of the bird and can be expected to improve productivity (Wang *et al*., 2000; Tizard, 2002). Immune proteins in broilers are dependent on the bursa of Fabricius and other related lymphatic organs and thymus which are essential for the transformation of IgM to IgG or functional IgA (Bienenstock *et al*., 1973). Therefore, increased production of cells containing immune proteins and increased blood concentration of immune proteins can be considered as a reciprocating act of lymphatic organs found in broilers fed with MCI. Zhang *et al*. (2003) reported a significant increase of the thymus index in broilers fed with IMO, consistent with the present results of the study.

Cecal microorganisms: The changes in the cecal content of microorganisms in broilers fed with MCI are shown in Table 3. The contents of the beneficial intestinal bacteria Bifidobacteria and Lactobacillus were higher in T3 and T4 compared with T1 and T2.

The contents of the harmful *E. coli* and Salmonella were significantly lower in T3 and T4 than in T1 and T2. The counts of lactic acid bacteria and *Bifidobacteria* in the cecal contents were higher in broilers in MCI given groups T3 and T4. This may have occurred because most of the encapsulated MCI passed through the stomach and small intestine, dodging hydrolysis and absorption, finally reaching the caecum where the MCI were dissolved and used as a substrate for the growth of both bacterial types (Gong *et al*., 2002; Park, 2008). Prebiotics such as inulin, oligosaccharide and oligosaccharide-related carbohydrates are not dissolved in the small intestine of animals and most (80%) reaches the large intestine where it reduces the counts of harmful microorganisms *E. coli*, Salmonella and Campylobacter and selectively promotes the proliferation of beneficial Bifidobacterium (Gibson and Wang, 1994; Gibson *et al*., 1995). Rada *et al*. (2001) and Rebole *et al*. (2010) reported that Bifidobacteria in caecum in broilers and laying hens increased significantly after administering inulin which supports the result of the study.

The importance of microorganisms in the alimentary tract is underlined by their actions in the synthesis of fermentation products that supply the visceral epithelial cells with necessary energy, stimulation of the immune system in the alimentary tract, synthesis of vitamin K and the resistance against the colonization of the extrinsic pathogenic microorganisms. While some microorganisms such as Lactobacillus and Bifidobacteria are beneficial for animal health, others like *E. coli* and *C. perfrigens* can be harmful (Devaraj *et al*., 2002). Since the bacterial flora in the intestine which include Bifidobacteria and Lactobacillus, compete with potential pathogenic microorganisms for nutrients and space for anchorage they suppress the colonization of pathogenic

microorganisms in the intestine. Also, Bifidobacteria and Lactobacillus secrete active bacteriocin that repels *E. coli* and Bifidobacteria produce organic acid and substrate that are repellent to other microorganisms. Most of the organic acids produced from fermentation by Lactobacillus are lactic acid and acetic acid. These substrates can suppress the colonization of pathogenic microorganisms (Gibson and Wang, 1994; Rolfe, 2000; Zhang *et al*., 2003). These mechanisms may contribute, at least in part to the significant reduction in the counts of *E. coli* and Salmonella in the caecum in broiler fed a diet containing MCI. The present result confirms the previous results (Xu *et al*., 2003) that in broilers whose diet included oligofluctiose, the counts of total anaerobic bacteria, Bifidobacteria and Lactobacillus in ceecum increased considerably while the count of *E. coli* decreased significantly. The caecum is the popular place of colonization in broilers for Salmonella which cause an infection that includes diarrhea and serious weight loss in broiler chicks. Low counts of *E. coli* and Salmonella in broilers fed with MCI is related to the significantly increased counts of Bifidobacteria and Lactobacillus. The feeding of MCI to broilers as a dietary supplement selectively stimulated the growth of Bifidobacteria and Lactobacillus in the caecum while suppressing the proliferation of harmful microorganisms. Also, the observations that the growth of beneficial Bifidobacteria and Lactobacillus in the caecum was promoted and the weight of immunologic organs increased in birds consuming MCI as compared to broilers fed a diet containing antibiotics group and an unsupplemented diet (Table 4) supports the view that MCI are potential bacterial growth suppressors that can be used to prevent diarrhea and promote growth in broiler chicks (Modler *et al*., 1990).

CONCLUSION

In this sudy, a concentration of 200 g ton^{-1}, microencapsulated-inulin in broiler diets can selectively increase the colony counts of the beneficial microorganisms, Bifidobacteria and Lactobacillus in the caecum and reduce the count of harmful *E. coli* and Salmonella, in contrast to broilers consuming an unsupplemented diet or feed supplemented with an antibiotics. Also, it was found that this preparation of an microencapsulated-inulin enhanced diet could improve the health and promote the growth performance of broilers by stimulating the proliferation of cells in the thymus, spleen and bursa of Fabricius which are major immune organs thus increasing the blood concentration of immune proteins.

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