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Abstracts

Physiological effects of polyphosphates with different chain lengths were unknown in poultry. The effect of 0.05% concentration of SCPP (short chain polyphosphates), MCPP (medium chain polyphosphates) and LCPP (long chain polyphosphates) was observed in broilers. MCPP and LCPP produced bacteriostatic properties against four pathogenic bacteria, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Salmonella enterica* ser. Pullorum, and *E. coli* O157:H7. SCPP reduced the level of triglycerides in the blood. Intervention of MCPP and LCPP induced cecum IL-1β expression involved in the regulation of autoimmune inflammation. In terms of colony-forming units, SCPP increased the number of *Lactobacilli* while MCPP and LCPP significantly decreasing the number of *Shigella*, *Salmonella* and *Coliform* bacteria. SCPP, MCPP, and LCPP improved the intestinal microflora with abundance of beneficial bacteria such as *Faecalibacterium*, *Phocaeicola*, and *Barnesiella* but with reduced *Bacteroides*. In addition, SCPP, MCPP, and LCPP did not adversely affect the meat quality of broilers. The antimicrobial properties of SCPP, MCPP, and LCPP can help to improve the intestinal environment and enhance immune properties. Based on the comparison of different length polyphosphates in broiler chickens, it is suggested that MCPP is more effective compared to SCPP and LCPP as antimicrobial feed additives.

Keywords: Polyphosphates, Antimicrobial activity, Anti-inflammation, Broilers, Microbiota

Introduction

The kind of linear polymer known as polyphosphates (Poly-p) is composed of tens or hundreds of orthophosphate (Pi) residues joined by high-energy phosphonic anhydride bonds. Poly-p are commonly found in cells. Since it was impossible to precisely measure or analyze the concentration of Poly-p in biological sources, their physiological roles were frequently disregarded in the past. Nowadays, it has been found that Poly-p possess diverse biological functions [1]. They are chelating agents for metal ions, buffers against alkalis, and capsules for bacteria. They play important roles in the processing and degradation of mRNA and environmental remediation of sodium phosphate depending on the requirement and location (species, cell, or subcellular compartment) [0,오류! 참조 원본을 찾을 수 없습니다.]. Poly-p have significant prohemostatic, pro-thrombotic, and pro-inflammatory effects, and long chain Poly-p are potent pro coagulant physiological activators and can act as modulators of coagulation and inflammation [3]. Poly-p are widely known for their role in biomedicine [오류! 참조 원본을 찾을 수 없습니다.]. Poly-p released by platelets or microorganisms will aid the expression of coagulation and fibrinolytic factors during wound healing [5]. In addition, Poly-p promote regeneration and repair of bone tissue [오류! 참조 원본을 찾을 수 없습니다.].

The biological chain length of Poly-p is variable and can be higher than 1000 n or lower than 100 n. The chemical effect of Poly-p with different chain lengths varies depending on the organism and the concentration of Poly-p used.; in general, the higher the value of the Poly-p polymer, the greater the inhibition of the growth of undesirable microorganisms [6]. There is scientific evidence showing that long chain Poly-p can be cut into multiple short chain Poly-p [6]. In heart therapy, two Poly-p with different chain lengths expressed differential effects [오류! 참조 원본을 찾을 수 없습니다.]. It has been found that medium and long chain Poly-p, consisting of an average of 60 phosphate residues, enhance cell proliferative activity in vitro, whereas short chain Poly-p, consisting of an average of 14 residues, have no such activity. Medium chain Poly-p influenced the creation and regeneration of bone, whereas long chain Poly-p had a strong inhibitory effect on bone resorption. Short chain Poly-p had little effect [10].

Poly-p have been used in animal feeding. Addition of Poly-p to dairy cow diets improves milk production efficiency [11]. Poly-p could improve the antioxidant properties of the organism as well as

54 the pH value of livestock products [12,13]. The addition of urea or ammonium Poly-p to rations fed to
55 pigs can increase serum urea nitrogen levels and ammonium polyphosphate has been used as a source of
56 phosphorus [14]. Previous experimental results have demonstrated several benefits of Poly-p for
57 broilers. Moon et al. [15] found that long chain Poly-p enhance the immune status based on broiler
58 growth, which includes improved growth performance, organ development, blood and intestinal
59 microflora constitution.

60 In previous experiments, the focus was only on the study of long or short chain Poly-p, while
61 comparisons of antimicrobial properties among short, medium, and long chains were rare. Therefore,
62 we conducted comparative analyses of in vitro antimicrobial properties, organ development, meat
63 quality, serum, anti-inflammatory and gut flora properties using SCPP, MCPP, and LCPP.

Materials and Methods

Preparation of experimental additives

RegeneTiss (Kunitachi, Japan) provided sodium Poly-p ($Na_{n+2}P_nO3_{n+1}$) with an average chain length of P3 (SCPP: short chain Poly-p), P14 (MCPP: medium chain Poly-p) and P130 (LCPP: long chain Poly-p) for the experiment.

Experimental animals and design

Forty 1-day-old Ross 308 male chicks were randomly divided into four groups of 10 chicks each, and 10 chickens were placed in a pen (1.8m long, 1.5m wide, 1.3m high): NC group (basal diet); P3 group (basal diet + 0.05% short Poly-p); P14 group (basal diet + 0.05% medium Poly-p); P130 group (basal diet + 0.05% long Poly-p). The basal diet was formulated according to the NRC [**오류! 참조 원본을 찾을 수 없습니다.**]. SCPP, MCPP and LCPP were dissolved in water (1 L) at 0.05% of the diet and premixed with a small amount (2 kg) of the basal diet. Afterwards, the premixed feed is mixed with the total feed for 30 minutes using a feed mixer to achieve a thorough mix (DKM-350SU, DAE KWANG, Hwaseong, Korea). The experimental period was divided into two phases, including a starter phase and a grower phase by basal diet formulations (Table 1). The feeding floor was covered with bran at a thickness of 5 cm. Feed and water were supplied throughout the entire trial, providing 23 hours of light and one hour of darkness. The temperature was set at 33°C for the first week and then lowered by two degrees per week until the end of the 22°C feeding period. At the end of the experiment, three broilers were randomly selected from each group, processed using animal ethics committee standards and samples were collected.

Antibacterial activity of Poly-p

Escherichia coli O157:H7 ATCC 35150, *Listeria monocytogenes* KCCM 40307, *Salmonella entericaser*. Gallinarum ATCC 9184, *Salmonella entericaser*. Pullorum SP4, *Pseudomonas aeruginosa* PA01, and *Shigella sonnei* KCTC 2518 were provided by Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). *Klebsiella pneumoniae* was from our laboratory

stock. *Escherichia coli* O157:H7, *S. entericaser*. Gallinarum, *S. entericaser*. Pullorum SP4, *K. pneumoniae*, and *S. sonnei* were grown in Luria-Bertani (LB; Difco, Franklin Lakes, NJ, USA) broth. *L. monocytogenes* and *P. aeruginosa* were grown in brain heart infusion (BHI, Difco) broth and nutrient broth (Difco), respectively. At 37°C with shaking (100 rpm), cultures were cultivated aerobically for 16 hours. LCPP, MCPP, and SCPP were dissolved in sterile distilled water to achieve a concentration of 50 mg/ml. Disinfect the solution by filtration by adjusting the pH to 7.0. Using sterilized cotton swabs, all freshly created bacterial cultures (~$1\times10^{8-9}$ CFU/mL) were swabbed onto corresponding agar plates. Aliquots (100 µL) of SCPP, MCPP, and LCPP at three same concentrations were then added into wells (6 mm in diameter) of agar plates. It was then incubated at 37°C for 16 hours, after which the zone of inhibition was observed, measured with a straightedge and recorded.

Organ

Breast meat, right leg, liver, spleen, bursa, small intestine (duodenum, jejunum and ileum) and cecum were collected from broilers. The weights were weighed using an electronic balance (EL4002, Mettler Toledo, Columbus, OH, USA). The length of the small intestine and cecum was subsequently measured and recorded using a tape measure. Units are expressed as a rate per 100 grams of live weight.

Meat quality

The measuring needle of the pH meter (Hanna Instruments, Nusfalau, Romania) was inserted 1 cm into the chicken breast, the value was read and three measurements were averaged. Cooking loss was calculated by heating samples in polyethylene bags in a water bath (C-WBE, Chang Shin co, Korea) at 75°C for 30 minutes. Chicken breasts were cooled for 10 minutes. The difference in weight of the chicken breasts before and after steaming was used to calculate the cooking loss using the following formula:

* Cooking loss (%) = (Sample weight before cooking - Sample weight after cooking) / (Sample weight before cooking) × 100

Flesh colour was measured on the surface of the samples using a colourimeter (Chromameter, CR210, minolta, Japan) with L* values for brightness, a* values for redness and b* values for yellowness. The standard colour used was a calibration plate with an L* value of 97.69, an a* value of -0.43, and a b* value of +1.98.

Serum

The chemical compositions of the blood samples taken from broilers in this experiment were analysed using an automated dry chemical analyser for veterinary use (CHEM7000i, Tokyo, Japan) at the Biological Center of Konkuk University Research Facility (Gwangjin-gu, Seoul, Korea).

Anti-inflammation

Each cytokine and beta actin gene accession number were obtained from NCBI reference sequence. MMLV-RT (Beams Bio, Korea) was used for converting 2μg of total RNA to first strand cDNA and then PCR reaction was performed at 94°C for 45 sec, 70°C for 2 min, and 55°C for 1 min for 30 cycles. The expected size of PCR product was indicated by arrow.

Expression levels of four different avian pro- or anti-inflammatory cytokine genes, interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1RN), IL-6, and tumor necrosis factor (TNF-α), in three intestinal organs (jejunum, ileum, and cecum) after feeding three different polymers (SCPP, MCPP, and LCPP) were examined with RT-PCR. Reverse transcription polymerase chain reaction (RT-PCR) was performed with an annealing temperature of 55℃ and 30 cycles of amplificatnion using an EmeraldAmp PCR Master Mix (Takara, Shiga Japan) in 10 μl volume. Forward and reverse primers for different cytokines are shown in Table 2. All primers were obtained from Cosmogentech (Seoul, Korea). The RT-PCR sample (10 μl) was loaded into each lane of a 1% ethidium bromide agarose gel and then visualized with UV Transilluminator from VILBER LOURMAT (Marne-la-Vallée cedex 3, France). Use ImageJ to measure protein grey values.

Microflora change on cecum

Three broilers were randomly taken from each group for sampling, totalling 12 samples. After cutting off the cecum, it was immediately stored in ice and transported back to the laboratory for microbiological enumeration. Intestinal contents were collected into sterile test tubes (50 ml) in a sterile environment. The number of surviving bacteria was counted within 24 hours using standard agar culture methods on deMan Rogosa Sharpe (MRS; Difco), nutrient broth, MacConkey (Difco), and *Streptococcus thermophilus* (ST) standard agar plates. Coliforms and lactose-negative enterobacteria

on MacConkey agar, total bacteria on normal nutrient agar, lactic acid bacteria (LAB) on MRS agar, and streptococci on ST agar were counted. All intestinal contents after treatment were sampled. Subsequently, 1g of each sample was serially diluted with sterilised distilled water. After being evenly distributed throughout the prepared medium, the diluted suspension was placed there and allowed to incubate for 24 to 48 hours at 37°C. Colonies were enumerated and represented as log CFU/g following incubation.

The previously reported procedures were followed for the PCR conditions, DNA extraction, bioinformatics, and NGS sequencing analysis [16]. In short, a PowerSoil DNA isolation kit (Mobio Laboratories, Inc., Carlsbad, CA, USA) was used for isolating genomic DNAs. Using 341F and 785R primers, the V3–V4 region of the bacterial 16S rRNA gene was amplified. Using an Illumina MiSeq platform through a Macrogen (Seoul, South Korea) commercial service, sequencing was done.

Statistical analysis

Data were examined in a completely randomized design using SAS 9.4's PROC mixed process (SAS Institute, Cary, NC, USA). Differences in means among treatment groups were determined using Tukey's test. Data variability was expressed as pooled standard error of mean (SEM). $p<0.05$ indicates statistical significance. $^{a-d}$ mean within a column within a main effect are significantly different.

Results

Antibacterial activity of Poly-p

Seven pathogenic bacteria harmful to poultry (*Listeria monocytogenes, Shigella sonnei, Salmonella enterica* ser. Gallinarum, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* ser. Pullorum, *Escherichia coli* O157:H7) were tested for antibacterial activity (Table 3). SCPP did not exhibit bacteriostatic activity. While MCPP and LCPP produced inhibitory effects on four pathogens including *Shigella sonnei, Pseudomonas aeruginosa*, *Salmonella enterica* ser. Pullorum, and *Escherichia coli* O157:H7. It is worth noting that MCPP presents a higher diameter of the inhibition circle than that of LCPP, so the inhibitory activity of MCPP is higher than that of LCPP.

Effects of SCPP, MCPP, and LCPP on organs

The effects of SCPP, MCPP, and LCPP on organ are shown in Table 4. The intervention of SCPP showed slightly reduced tendency of liver weight and increased jejunal length compared to the control NC group ($p<0.05$). LCPP showed reduced tendency of liver weight ($p<0.05$) and slightly increased jejunal length compared to the control group.

Effects of SCPP, MCPP, and LCPP on meat quality

In meat quality (Table 5), SCPP, MCPP, and LCPP did not show any effects except for the decreased lightness of MCPP at cooking loss ($p<0.05$). However, LCPP increased it compared to MCPP.

Effects of SCPP, MCPP, and LCPP on serum

Effects of LCPP, MCPP, and SCPP on serum are shown in Fig. 1. Results showed that LCPP increased the level of glucose (Fig. 1d) in the body ($p<0.05$). SCPP decreased the level of triglycerides (Fig. 1g) in vivo compared to control NC, whereas LCPP increased the level of triglycerides (both $p<0.05$).

Effects of SCPP, MCPP, and LCPP on anti-inflammation

Poly-p affected the expression of pro-inflammatory cytokines in the intestine (Fig. 2a). All treatment groups showed lower IL-1β expression in the ileum and jejunum, while polymers MCPP and LCPP enhanced IL-1β expression in the cecum. More interesting to note that while IL-6 and TNFα were not expressed in these tissues, the anti-inflammatory cytokine IL-1RN was expressed constitutively at extremely low levels (Figs. 2b, c, d).

Microflora change on cecum

According to the CFU of bacteria count statistics (Table 6). SCPP was able to increase the abundance of *Lactobacillus* in the cecum and decrease the abundance of coliform bacteria. Subsequently, MCPP and LCPP also reduced the abundance of coliform bacteria and *Shigella* and *Salmonella* in the cecum compared to control NC.

Table 7 shows the alpha-diversity indices (ASVs, Chao1, Shannon, and Gini-Simpson) for the gut microbiota of broilers ingesting SCPP, MCPP, and LCPP. In the P14 group, the Asvs and Chao1 indices were significantly stronger than in the NC group ($p<0.05$). Regarding beta diversity (Figs. 3g and h), the PCoA plots with weighted Unifrac distances clearly showed that the microbial colonies formed confidence zones between groups, and the confidence triangles of the bacterial communities in the P130 treatment group area deviated significantly from those of the NC group. Meanwhile, PD_whole_tree (UPGMA) showed comparable species homology between the other treatment groups and the NC group.

To determine the changes in the gut flora after SCPP, MCPP, and LCPP interventions, the microbiological components were analysed. The microflora in the cecum of broiler chickens at the phylum level mainly consisted of *Bacteroidetes* (31.4%) and *Firmicutes* (65.01%). Two superior species, Bacteroidetes and Firmicutes, accounted for ~95% of the total microorganisms in relative abundance (Figs 3a, Table 8).

Figure 3b depicts the classification components of the gut flora at the genus level. *Phocaeicola* (15.23%) and *Mediterraneibacter* (8.59%) stood out from the rest of the microflora, and these two bacteria accounted for the largest percentage. Four dominant bacterial families were chosen to analyse the variations in the gut microbiota compositions in various samples (Figs. 3c-f, Table 8). The relative abundance of some *Bacteroides* was significantly lower in groups P3 and P14 compared to the control NC. However, the abundance of *Phocaeicola* and *Faecalibacterium* were higher in the P130 group

than in the control NC, and the abundance of *Barnesiella* were higher in the P14 group than in the control NC (both $p<0.05$).

Discussion

A polyphosphate derivative was found to have higher antibacterial activity against Gram-positive bacteria than against Gram-negative bacteria [15]. There is a possibility that Poly-p can isolate metal ions to stabilize, thus reducing the availability of nutrients to cells and making cells gradually become apoptotic [16,6]. In the presence of long chain Poly-p, cell envelopes of *Staphylococcus aureus* and *Bacillus cereus* are damaged. It is worth stating that 0.05% Poly-p inhibits spore germination and growth, while high concentrations (1.0%) of Poly-p can even kill spores [19,20]. On the other hand, certain gram-negative bacteria appear to be resistant to antibacterial properties of Poly-p, and none of the high concentrations seemed to have an effect [21,**오류! 참조 원본을 찾을 수 없습니다.**]. Notably, compared to gram-negative bacteria, gram-positive bacteria have far higher requirements for Mg^{2+}, which may be one of the reasons why gram-positive bacteria are more sensitive to Poly-p [**오류! 참조 원본을 찾을 수 없습니다.**]. Results of the present study, MCPP and SCPP inhibited the growth performance of most of the pathogenic bacteria.

Liver weight is generally considered to be proportional to body weight 24. Although Moon et al. [15] concluded that long-chain Poly-p at a concentration of 0.1% would not affect the liver of broilers. But, high (1.0%) or low (0.1%) dietary inorganic phosphate intake can negatively affect liver development in mice, another study reported that adding 10% sodium trimetaphosphate to the diet of mice resulted in disrupted liver growth and development 2526. This experiment will not exclude the possibility of liver-induced toxic reactions at certain doses. It is widely recognised that an increase in the length of the gut may not be a good thing. The reduction and enlargement of chicken intestinal volume may reflect the organism's nutrient absorption capacity and utilisation efficiency 27. When long-chain Poly-p are added, the jejunum, ileum, and cecum get shorter and lighter [15].

For broiler carcass quality assessment, meat brightness, redness and pH are considered 28. Poly-p are used as antioxidants in food products 29. In this experiment, SCPP, MCPP, and LCPP did not affect the meat quality of broilers except for the lightness at cooking loss. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat were influenced by concentration of sodium

tripolyphosphate 29.

The ability of broilers to deposit fat is correlated with triglycerides, it is a class of neutral lipids that is essential to the body's ability to produce cells, metabolize them, and use them as a source of energy 3132. From the results, the increase in triglycerides levels and glucose levels did not affect the broiler's own body weight.

Poly-p activities are dependent on chain length. Phosphate polymerization may therefore be essential for promoting an inflammatory response. When a Poly-p chain included more than 65 monomers, its effects on lipopolysaccharide-induced macrophage inflammation were more pronounced, and previous results have shown that Poly-p-amplified lipopolysaccharide could induce inflammatory responses of macrophages, which provides a new therapeutic target for inflammatory diseases 33. High levels of Poly-p can reduce the ability of neutrophils and macrophages to phagocytose bacteria and decrease the expression of macrophage attracting chemokines (such as CCL2 and CXCL10) and activating interferon beta in a Poly-p dose and chain length dependent manner 34. Cytokines of the interleukin-1 receptor antagonist (IL-1RN) and interleukin-1b (IL-1β) are essential in controlling the inflammatory responses of the gastrointestinal mucosa 35.

Poly-p bring the internal intestinal flora into balance [6]. *Lactobacillus* are gram-positive bacteria that are healthy for the intestinal tract 36. It has been found that 700 pi chain length of Poly-p accumulated in *Lactobacillus paracasei* is effective in promoting a healthy gut 37. There are a wide variety of coliform bacteria, *Shigella*, and *Salmonella* most of which can have a direct impact on gut health 3839. In this result, SCPP, MCPP and LCPP all increased the number of beneficial bacteria in the intestinal tract and controlled the number of harmful bacteria, which improved the structure of the intestinal environment and promoted the nutrient absorption of the organism.

The intestinal microbiota profoundly influences intestinal homeostasis, not only affecting intestinal metabolites but also regulating intestinal immune homeostasis 40. The study of alpha and beta indices was analysed herein, with the results showing that SCPP, MCPP, and LCPP increased the homology and diversity of microorganisms in the cecum, with MCPP being the most effective. At the phylum level, Bacteroidetes and Firmicutes were above 95%. SCPP and MCPP reduced the abundance values of some of the *Bacteroides*. A significant proportion of the *Bacteroides* were harmful species, such as *Bacteroides vulgatus* and *Bacteroides fragilis*, both of which are commonly associated with cases of inflammation and abscesses 41. Subsequently, LCPP can increase the abundance of *Phocaeicola* and

Faecalibacterium. Phocaeicola's metabolite (3-Hydroxyphenylacetic acid) can alleviate fatty liver disease associated with metabolic dysfunction and is a beneficial bacterium 42. *Faecalibacterium* is a butyrate producer and has been shown to possess anti-inflammatory properties both in vivo and in vitro, with the potential to be a key member of gut microbiota homeostasis 43. SCPP increased the abundance of *Barnesiella*. *Barnesiella* is a valuable microorganism that can help cyclophosphamide for tumour immunosurveillance 44. Therefore, it is suggested that the intervention of SCPP, MCPP, and LCPP changes the structure of the intestinal flora, increases its diversity, promotes the growth of beneficial bacteria in the intestinal tract, and inhibits the growth of harmful bacteria.

Conclusion

In summary, SCPP, MCPP, and LCPP all have antibacterial properties, can promote anti-inflammatory properties, improve intestinal microflora and serum status, and the effect is comparable to antibiotics. Among the three Poly-p with different chain lengths, MCPP has better effect than SCPP and LCPP. More importantly, SCPP, MCPP, and LCPP have no toxic side effects and can be an important basis for the use of antimicrobial feed additives for poultry.

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Tables and Figures

Table 1. Nutritional compositions of the basal diet

Items	Starter （1 to 21 day）	Grower （21 to 35 day）
Ingredient, %		
Corn	50.75	53.511
Wheat	5.00	5.00
SBM (IMP)	34.09	31.331
Tallow	4.99	6.117
L-methionine, 98%	0.32	0.247
Lysine-Syn 24%	0.96	0.244
L-threonine, 98%	0.13	0.027
Limestone	1.59	1.535
MDCP	1.29	1.156
Choline Cl, 50%	0.10	0.071
Salt	0.28	0.28
Vitamin Premix[a]	0.15	0.15
Mineral Premix[b]	0.15	0.15
Phytase	0.02	0.02
NaHCO3	0.16	0.16
Chemical composition calculated		
CP, %	21.00	19.00
Crude fiber, %	2.88	2.96
Ca, %	0.90	0.85
Available Phosphorus, %	0.35	0.32
Total Lys, %	1.37	1.10
Total TSAA, %	0.99	0.87
Total Thr, %	0.90	0.74
AMEn, kcal/kg	3,050	3,146

[a]Per kilogram of diet, mineral premix contained the following: 80 mg Fe; 50 mg Zn; 60 mg Mn; 0.3 mg Co; 10 mg Cu; 0.2 mg Se.

[b]Per kilogram of diet, vitamin premix ingredients were as follows: vitamin A, 80,000 IU; vitamin D3,1600 IU; vitamin E, 20 IU; vitamin K3, 8 mg; vitamin B1, 8 mg; vitamin B2, 24 mg; vitamin B6, 12 mg; vitamin B12, 0.040 mg; pantothenic acid, 40 mg; folic acid, 4 mg; nicotinic acid, 120 mg. Met, formethionine; Cys, cysteine; and AMEn, apparent metabolizable energy.

429 **Table 2.** Primers for cytokines measurement

Gene[1)]	Forward primer	Reverse primer
β-actin	ACCAACTGGGACGACATGGA	GTGATGACCTGGCCGTCAG
IL-1β	AGAGATGGCGTTCGTTCCC	GCAGTCAGCGCCCACTTA
IL-1RN	ATTGGGGCATCTCATGGGTG	GCTCAGCACAGCTGGAAGTA
IL-6	AGAAGCCGCACCATGAACTT	TGGTAACAGAGGATTGTGCCC
TNFα	ATGACCACGCTCTTTCCGT	TTAATCCACTCCCACCACCC

430 [1)]β-actin, Beta-actin; IL-1β, Interleukin-1 beta; IL-1RN, Interleukin 1 receptor antagonist; IL-6, Interleukin 6; TNFα, Tumor
431 necrosis factor.

432

433 **Table 3.** Antibacterial effect of different length Polyphosphates for pathogenic bacteria

Strain	Inhibition zone (mm)[1)]		
	P3	P14	P130
Listeria monocytogenes	ND[2)]	ND	ND
Shigella sonnei	ND	20.76±0.09	15.99±0.62
Salmonella enterica ser. Gallinarum	ND	ND	ND
Klebsiella pneumoniae	ND	ND	ND
Pseudomonas aeruginosa	ND	11.00±0.65	9.89±0.74
Salmonella enterica ser. Pullorum	ND	13.78±0.15	10.20±0.20
Escherichia coli O157:H7	ND	10.70±0.30	9.56±0.89

434 [1)]SCPP, short chain Polyphosphates; MCPP, Medium chain Polyphosphates chain Polyphosphates; LCPP, long chain
435 Polyphosphates.
436 [2)]ND; not detected.

437

438 **Table 4.** Effect of different length Polyphosphates for tissue and organ

Response parameter	Treatment[1)]				SEM[2)]	*p-value*
	NC	P3	P14	P130		
Weights (g)						
Body weight	1753	1794	1883	1724	0.04	0.0684
Liver	2.06[a]	1.84[ab]	1.93[a]	1.56[b]	0.07	0.0079
Spleen	0.11	0.11	0.09	0.08	0.02	0.5453
Fabricius of bursa	0.17	0.14	0.14	0.11	0.03	0.4099
Breast	9.80	9.83	10.70	10.41	0.39	0.3340
Single leg	6.05	6.07	5.76	6.20	0.16	0.3303
Length (cm)						
Duodenum	26.87	30.73	25.00	29.50	1.86	0.2046
Jejunum	62.93[b]	79.00[a]	65.47[ab]	71.47[ab]	3.16	0.0288
Ileum	62.50	71.10	65.30	75.07	4.42	0.2562
Cecum	16.70	18.74	17.47	19.17	0.89	0.2557
Length to body weight ratio（%）						
Duodenum	1.59	1.59	1.41	1.61	0.17	0.8391
Jejunum	3.71	4.07	3.70	3.87	0.34	0.8498
Ileum	3.68	3.65	3.68	4.08	0.37	0.8236
Cecum	0.99	0.97	0.98	1.04	0.09	0.9515

439 [1)]NC, basal diet; P3, basal diet + 0.05% short chain Polyphosphates; P14, basal diet + 0.05% medium chain Polyphosphates;
440 P130, basal diet + 0.05% Long chain Polyphosphates.
441 [2)]SEM: standard error of mean. $p<0.05$ indicates statistical significance.

Table 5. Effect of different length Polyphosphates for meat quality

Item[2)]	Treatment[1)]				SEM[3)]	*p-value*
	NC	P3	P14	P130		
pH	5.73	5.76	5.81	5.70	0.06	0.5718
Cooking loss (%)	17.16	17.07	15.63	20.10	0.99	0.0669
L*	60.35^{ab}	61.18^{ab}	59.50^{b}	62.00^{a}	0.55	0.0187
a*	1.66	2.25	1.72	1.81	0.19	0.1444
b*	2.52	2.95	2.60	3.11	0.38	0.6503

[1)]NC, basal diet; P3, basal diet + 0.05% short chain Polyphosphates; P14, basal diet +0.05% medium chain Polyphosphates; P130, basal diet + 0.05% long chain Polyphosphates.

[2)]L*, Lightness; a*, Redness; b*, Yellowness.

[3)]SEM: standard error of mean. $p<0.05$ indicates statistical significance.

449 **Table 6.** Effect of different length Polyphosphates for intestinal microorganisms

Item(logCFU/g)	Treatment[1)]				SEM[2)]	*p-value*
	NC	P3	P14	P130		
Total microbes	9.33^{ab}	9.60^{a}	9.28^{ab}	9.08^{b}	0.13	0.0486
Lactobacilli	9.02^{bc}	9.63^{a}	9.4^{ab}	8.67^{c}	0.15	0.0006
Coliform bacteria	8.79^{a}	8.35^{b}	7.96^{c}	8.14^{bc}	0.10	<0.0001
Shigella and Salmonella	8.67^{a}	8.40^{ab}	8.05^{b}	8.22^{b}	0.10	0.0008

450 [1)]NC, basal diet; P3, basal diet + 0.05% short chain Polyphosphates; P14, basal diet + 0.05% medium chain Polyphosphates;
451 P130, basal diet + 0.05% long chain Polyphosphates.
452 [2)]SEM: standard error of mean. $p<0.05$ indicates statistical significance.
453

454 **Table 7.** Effect of different length Polyphosphates for microbial alpha indicators of cecum

Item[2)]	NC[1)]	P3	P14	P130	SEM[3)]	*p*-value
ASVs	398[b]	388[b]	461.67[a]	397.67[b]	13.8	0.0187
Chao1	400.57[b]	388.23[b]	468.54[a]	398.64[b]	14.1	0.0136
Shannon	6.72	6.3	6.48	6.61	0.2	0.5247
Simpson	0.97	0.94	0.96	0.97	0.01	0.2455

455 [1)]NC, basal diet; P3, basal diet + 0.05% short chain Polyphosphates; P14, basal diet + 0.05% medium
456 chain Polyphosphates; P130, basal diet + 0.05% long chain Polyphosphates.
457 [2)]ASVs: bacterial amplicon sequence variants, Chao1: Community richness, Shannon: Number and
458 homogeneity of species, Simpson: Probability that any two individuals drawn from a community
459 belong to different species.
460 [3)]SEM: standard error of mean. $p<0.05$ indicates statistical significance.
461

462 **Table 8.** Effect of different chain length polyphosphates on phylum and genus in the intestinal flora

Item	Treatment[1)]				SEM[2)]	*p*-value
	NC	P3	P14	P130		
Phylum (%)						
Bacteroidetes	30	32	29	34	5	0.9196
Firmicutes	67	65	66	62	6	0.9274
Lentisphaerae	0.01	0.01	0.01	0.0067	0.009	0.9444
Proteobacteria	1.18	1.06	1.11	1.11	0.076	0.7246
Tenericutes	0.077	0	0.003	0.05	0.04	0.5005
Others	1.65	1.95	3.05	2.27	0.38	0.1257
Genus (%)						
Bacteroides	12.03^{a}	1.76^{b}	1.93^{b}	4.89^{ab}	2.18	0.0325
Phocaeicola	4.56^{b}	17.23^{ab}	17.88^{ab}	21.26^{a}	3.44	0.0388
Barnesiella	5.92^{b}	17.91^{a}	5.9^{b}	3.91^{b}	2.48	0.0145
Faecalibacterium	3.65^{b}	4.52^{ab}	4.16^{b}	10.67^{a}	1.42	0.0256
Streptococcus	3.07	6.01	5.93	2.06	2.75	0.6713
Eubacterium	0.07	0.07	0.21	1.37	0.33	0.0679
Blautia	4.66	2.37	4.21	5.32	0.94	0.2256
Lachnoclostridium	2.44	1.78	1.70	2.20	0.22	0.1375
Saccharofermentans	1.06	0.53	0.22	0.36	0.39	0.4788
Romboutsia	5.53	5.76	8.12	3.73	1.79	0.4352
Turicibacter	1.50	2.08	2.31	1.25	0.30	0.121
Ligilactobacillus	0.76	0.81	0.46	0.25	0.20	0.2292
Pseudescherichia	0.25	0.14	0.14	0.09	0.06	0.4213
Mediterraneibacter	8.86	9.91	8.32	7.25	1.64	0.7207
Others	41.51	39.64	42.10	40.06	2.39	0.8683

463 [1)]NC, basal diet; P3, basal diet + 0.05% short chain polyphosphates; P14, basal diet + 0.05% medium
464 chain polyphosphate; P130, basal diet + 0.05% long chain polyphosphate.
465 [2)]Values are presented as mean and standard error of mean (SEM).
466 [a-b)]in the same row, with significant differences indicated between values marked differently above (p
467 ≤ 0.05).
468

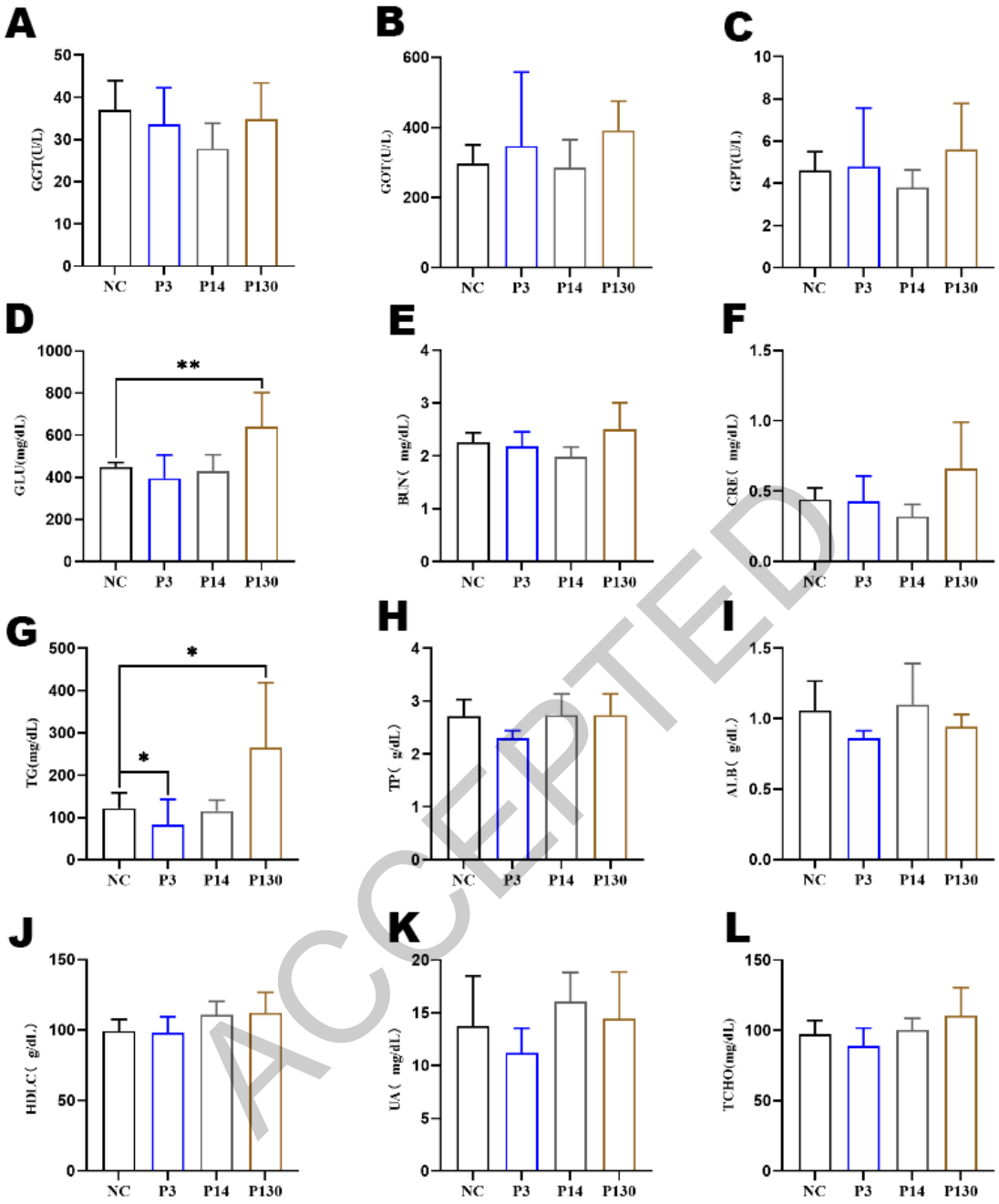

Fig. 1. Effect of different length polyphosphates for serum. (a) Gamma-glutamyl triglyceride (GGT). (b) Glutamate oxaloacetate transaminase (GOT). (c) Glutamate pyruvate transaminase (GPT). (d) Glucose (GLU). (e) Blood urea nitrogen (BUN). (f) Creatine (CRE). (g) Triglycerides (TG). (h) Total protein (TP). (i) Albumin (ALB). (j) High density lipoprotein (HDLC). (k) Uric acid (UA), (l) Total cholesterol (TCHO). *indicate statistical significance at $p<0.05$.

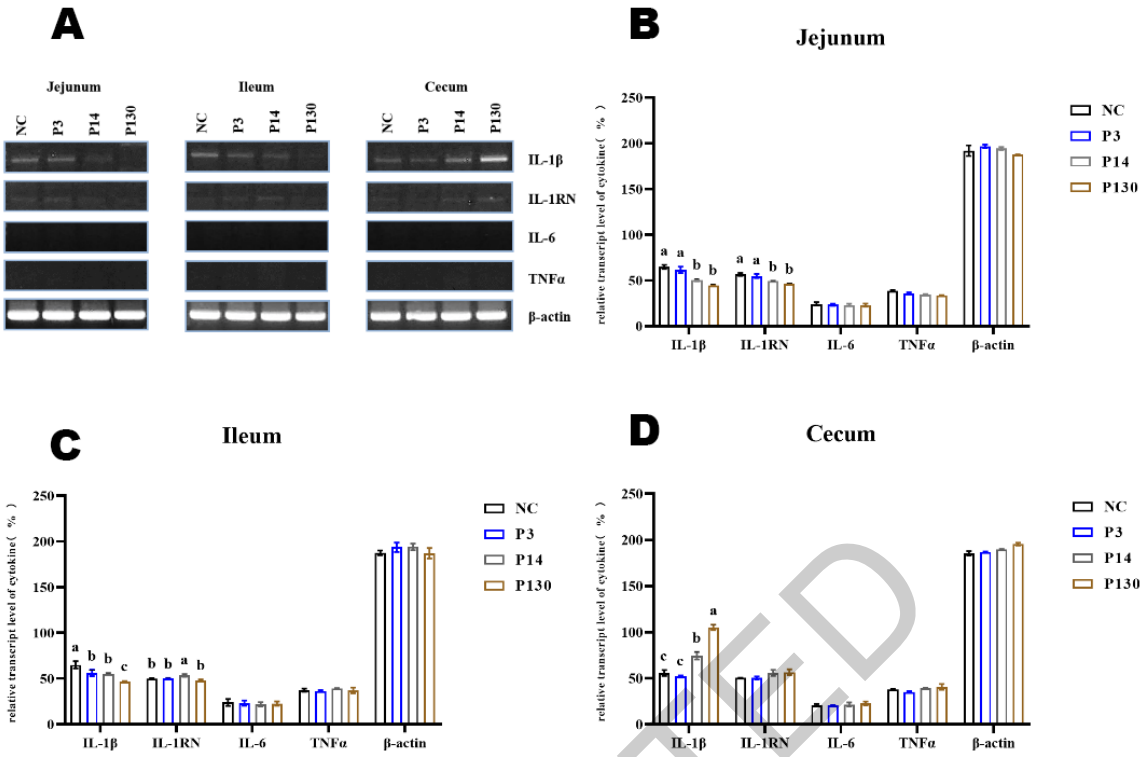

Fig. 2. Expression of four different avian pro-inflammatory cytokine genes (a), interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1RN), IL-6, and tumor necrosis factor (TNF-α), in three intestinal organs, jejunum (b), ileum (c), and cecum(d) after feeding three different polymers (P3, P14, and P130) were examined with RT-PCR.

NC = negative control, P3 = short chain polyphosphates (SCPP), P14 = medium chain polyphosphates (MCPP), P130 = long chain polyphosphates (LCPP). $^{a-d}$ mean within a column within a main effect are significantly different (p<0.05)

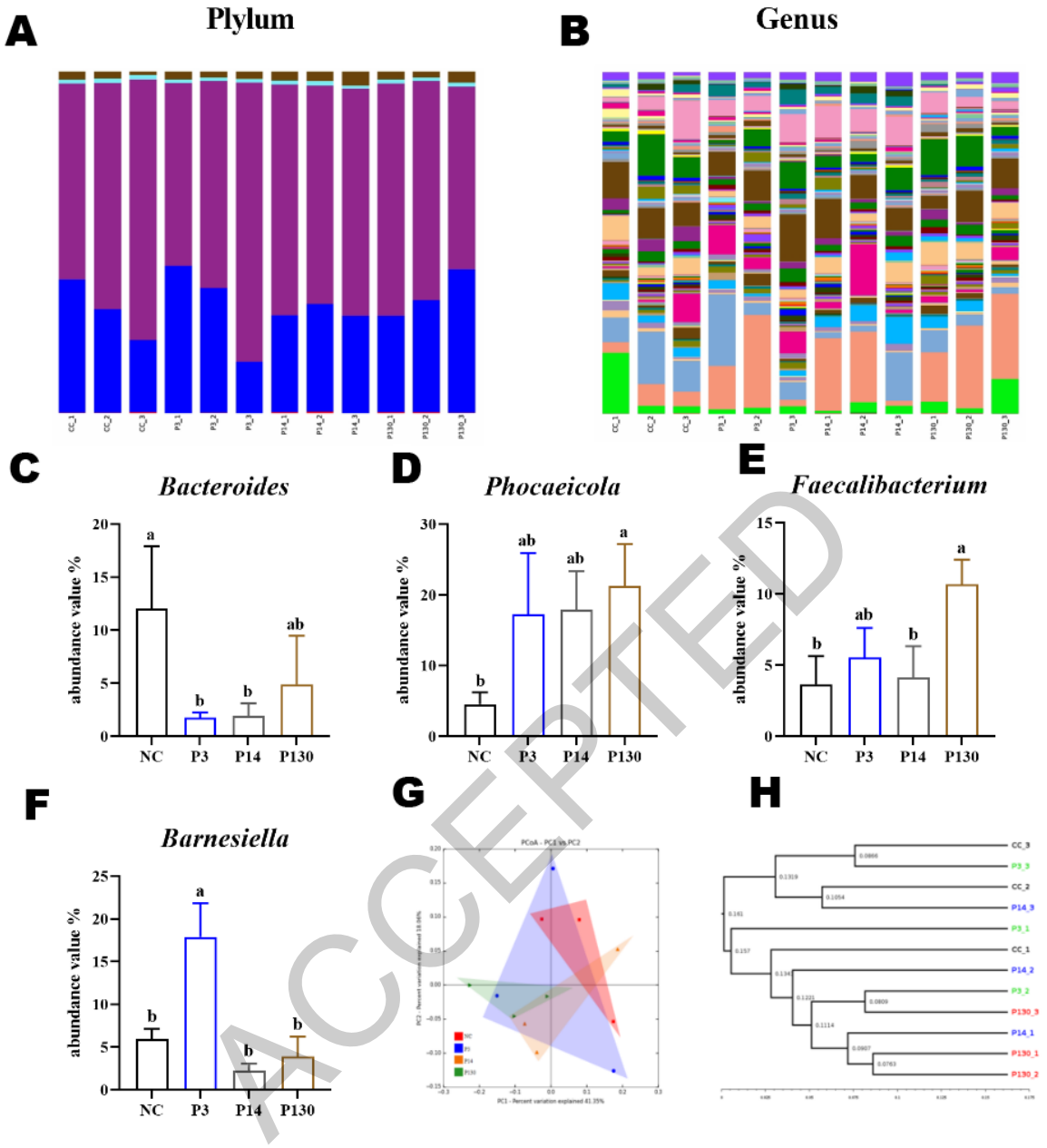

Fig. 3. Effect of different length polyphosphates for intestinal flora in cecum. (a) Phylum level. (b-f) Relative abundances of the gut microbiota at the genus level. (g-h) PCoA analysis based on Bray-Curtis distance and Phylogenetic tree of the cecum microbiota, where the confidence interval is 95%. $^{a-d}$ mean within a column within a main effect are significantly different (p<0.05, n=3).