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Article Title (within 20 words without abbreviations)	Effects of polyphosphates with different chain lengths on digestive organ weight, carcass quality, and immune response, and intestinal microflora in broilers
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Author	Yi-Qiang Chang ¹ , Seung-Gyu Moon ¹ , Yan-Qing Wang ¹ , Jong-Seok Oh ¹ , Sang-Woo Jeon ¹ , Ah-Ran Lee ² , Soo-Hyun Kim ³ and Soo-Ki Kim ^{1*}
Affiliation	¹ Department of Animal Science and Technology, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea ² Animal Resources Research Center, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea ³ Laboratory of Cytokine Immunology, College of Veterinary Medicine, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Yi-Qiang Chang (https://orcid.org/0000-0001-9732-1705) Seung-Gyu Moon (https://orcid.org/0000-0003-4953-4102) Yan-Qing Wang (https://orcid.org/0009-0006-5848-031X) Jong-Seok Oh (https://orcid.org/0009-0003-3068-7907) Sang-Woo Jeon (https://orcid.org/0000-0002-1414-8175) Ah-Ran Lee (https://orcid.org/0000-0001-7163-0059) Soo-Hyun Kim (https://orcid.org/0000-0002-8277-7424) Soo-Ki Kim (https://orcid.org/0000-0003-3499-3330)
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5 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Soo-Ki Kim
Email address – this is where your proofs will be sent	sookikim@konkuk.ac.kr
Secondary Email address	cyq123@konkuk.ac.kr
Address	Department of Animal Science and Technology, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea
Cell phone number	010 2965 3728
Office phone number	+82.2.4503728
Fax number	+82.2.4503728

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7 **Abstracts**

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

23

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

25

Introduction

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

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

52 Poly-p have been used in animal feeding. Addition of Poly-p to dairy cow diets improves milk
53 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.

64

65

Materials and Methods

66 Preparation of experimental additives

67 RegeneTiss (Kunitachi, Japan) provided sodium Poly-p ($\text{Na}_{n+2}\text{P}_n\text{O}_{3n+1}$) with an average chain length
68 of P3 (SCPP: short chain Poly-p), P14 (MCP: medium chain Poly-p) and P130 (LCPP: long chain
69 Poly-p) for the experiment.

70 Experimental animals and design

71 Forty 1-day-old Ross 308 male chicks were randomly divided into four groups of 10 chicks each,
72 and 10 chickens were placed in a pen (1.8m long, 1.5m wide, 1.3m high): NC group (basal diet); P3
73 group (basal diet + 0.05% short Poly-p); P14 group (basal diet + 0.05% medium Poly-p); P130 group
74 (basal diet + 0.05% long Poly-p). The basal diet was formulated according to the NRC [오류! 참조
75 원본을 찾을 수 없습니다.]. SCPP, MCP and LCPP were dissolved in water (1 L) at 0.05% of the
76 diet and premixed with a small amount (2 kg) of the basal diet. Afterwards, the premixed feed is
77 mixed with the total feed for 30 minutes using a feed mixer to achieve a thorough mix (DKM-350SU,
78 DAE KWANG, Hwaseong, Korea). The experimental period was divided into two phases, including a
79 starter phase and a grower phase by basal diet formulations (Table 1). The feeding floor was covered
80 with bran at a thickness of 5 cm. Feed and water were supplied throughout the entire trial, providing
81 23 hours of light and one hour of darkness. The temperature was set at 33°C for the first week and
82 then lowered by two degrees per week until the end of the 22°C feeding period. At the end of the
83 experiment, three broilers were randomly selected from each group, processed using animal ethics
84 committee standards and samples were collected.

85 Antibacterial activity of Poly-p

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

90 stock. *Escherichia coli* O157:H7, *S. entericaser. Gallinarum*, *S. entericaser. Pullorum* SP4, *K.*
91 *pneumoniae*, and *S. sonnei* were grown in Luria-Bertani (LB; Difco, Franklin Lakes, NJ, USA) broth.
92 *L. monocytogenes* and *P. aeruginosa* were grown in brain heart infusion (BHI, Difco) broth and
93 nutrient broth (Difco), respectively. At 37°C with shaking (100 rpm), cultures were cultivated
94 aerobically for 16 hours. LCPP, MCPP, and SCPP were dissolved in sterile distilled water to achieve a
95 concentration of 50 mg/ml. Disinfect the solution by filtration by adjusting the pH to 7.0. Using
96 sterilized cotton swabs, all freshly created bacterial cultures ($\sim 1 \times 10^{8-9}$ CFU/mL) were swabbed onto
97 corresponding agar plates. Aliquots (100 μ L) of SCPP, MCPP, and LCPP at three same concentrations
98 were then added into wells (6 mm in diameter) of agar plates. It was then incubated at 37°C for 16 hours,
99 after which the zone of inhibition was observed, measured with a straightedge and recorded.

100 **Organ**

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

105 **Meat quality**

106 The measuring needle of the pH meter (Hanna Instruments, Nusfalau, Romania) was inserted 1 cm
107 into the chicken breast, the value was read and three measurements were averaged. Cooking loss was
108 calculated by heating samples in polyethylene bags in a water bath (C-WBE, Chang Shin co, Korea) at
109 75°C for 30 minutes. Chicken breasts were cooled for 10 minutes. The difference in weight of the
110 chicken breasts before and after steaming was used to calculate the cooking loss using the following
111 formula:

112 * Cooking loss (%) = (Sample weight before cooking - Sample weight after cooking) / (Sample weight
113 before cooking) \times 100

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

118 **Serum**

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

122 **Anti-inflammation**

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

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

137 **Microflora change on cecum**

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

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

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

155 **Statistical analysis**

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

160

Results

161

162 **Antibacterial activity of Poly-p**

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

170 **Effects of SCPP, MCPP, and LCPP on organs**

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

175 **Effects of SCPP, MCPP, and LCPP on meat quality**

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

179 **Effects of SCPP, MCPP, and LCPP on serum**

180 Effects of LCPP, MCPP, and SCPP on serum are shown in Fig. 1. Results showed that LCPP
181 increased the level of glucose (Fig. 1d) in the body ($p<0.05$). SCPP decreased the level of triglycerides
182 (Fig. 1g) in vivo compared to control NC, whereas LCPP increased the level of triglycerides (both
183 $p<0.05$).

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

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

190 **Microflora change on cecum**

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

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

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

208 Figure 3b depicts the classification components of the gut flora at the genus level. *Phocaeicola*
209 (15.23%) and *Mediterraneibacter* (8.59%) stood out from the rest of the microflora, and these two
210 bacteria accounted for the largest percentage. Four dominant bacterial families were chosen to analyse
211 the variations in the gut microbiota compositions in various samples (Figs. 3c-f, Table 8). The relative
212 abundance of some *Bacteroides* was significantly lower in groups P3 and P14 compared to the control
213 NC. However, the abundance of *Phocaeicola* and *Faecalibacterium* were higher in the P130 group

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

216

217

Discussion

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

230 Liver weight is generally considered to be proportional to body weight [24]. Although Moon et al. [15]
231 concluded that long-chain Poly-p at a concentration of 0.1% would not affect the liver of broilers. But,
232 high (1.0%) or low (0.1%) dietary inorganic phosphate intake can negatively affect liver development
233 in mice, another study reported that adding 10% sodium trimetaphosphate to the diet of mice resulted
234 in disrupted liver growth and development [25,26]. This experiment will not exclude the possibility of
235 liver-induced toxic reactions at certain doses. It is widely recognised that an increase in the length of
236 the gut may not be a good thing. The reduction and enlargement of chicken intestinal volume may
237 reflect the organism's nutrient absorption capacity and utilisation efficiency [27]. When long-chain
238 Poly-p are added, the jejunum, ileum, and cecum get shorter and lighter [15].
239 For broiler carcass quality assessment, meat brightness, redness and pH are considered [28]. Poly-p are
240 used as antioxidants in food products [29]. In this experiment, SCPP, MCP, and LCPP did not affect the
241 meat quality of broilers except for the lightness at cooking loss. Variation in myoglobin denaturation
242 and color of cooked beef, pork, and turkey meat were influenced by concentration of sodium

243 tripolyphosphate 29.

244 The ability of broilers to deposit fat is correlated with triglycerides, it is a class of neutral lipids that is
245 essential to the body's ability to produce cells, metabolize them, and use them as a source of energy
246 3132. From the results, the increase in triglycerides levels and glucose levels did not affect the broiler's
247 own body weight.

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

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

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

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

281

282 **Conclusion**

283

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

289

290

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293

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References

295 1. Kornberg A. Inorganic Polyphosphate: toward making a forgotten polymer unforgettable. J
296 Bacteriol. 1995;177(3):491-496. <https://doi.org/10.1128/jb.177.3.491-496.1995>

297 2. Kumble KD, Kornberg A. Inorganic Polyphosphate in mammalian cells and tissues. J Biol Chem.
298 1995;270(11):5818-22. <https://doi.org/10.1074/jbc.270.11.5818>

299 3. Morrissey JH, Choi SH, Smith SA. Polyphosphate: an ancient molecule that links platelets,
300 coagulation, and inflammation. Blood. 2012;119(25):5972-9.
301 <http://doi.org/10.1182/blood-2012-03-306605>

302 4. Kulakovskaya TV, Vagabov VM, Kulaev IS. Inorganic Polyphosphate in industry, agriculture
303 and medicine: Modern state and outlook. Process Biochemistry. 2012;47(1):1-10.
304 <http://doi.org/10.1016/j.procbio.2011.10.028>

305 5. Smith S A, Mutch N J, Baskar D, et al. Polyphosphate modulates blood coagulation and
306 fibrinolysis. Proc Natl Acad Sci. 2006;103(4):903-908. <https://doi.org/10.1073/pnas.0507195103>

307 6. Wang Y, Li M, Li P, Teng H, Fan D, Du W, et al. Progress and Applications of Polyphosphate in
308 Bone and Cartilage Regeneration. Biomed Res Int. 2019;2019:5141204.
309 <http://doi.org/10.1155/2019/5141204>

310 7. Obritsch JA, Ryu D, Lampila LE, Bullerman LB. Antibacterial effects of long-chain
311 Polyphosphate on selected spoilage and pathogenic bacteria. J Food Prot. 2008;71(7):1401-5.
312 <http://doi.org/10.4315/0362-028x-71.7.1401>

313 8. Shi X, Kornberg A. Endopolyphosphatase in *Saccharomyces cerevisiae* undergoes
314 post-translational activations to produce short-chain Polyphosphates. FEBS Lett.
315 2005;579(9):2014-8. <https://doi.org/10.1016/j.febslet.2005.02.032>

316 9. Seidlmayer LK, Gomez-Garcia MR, Shiba T, Porter GA, Jr., Pavlov EV, Bers DM, et al. Dual
317 role of inorganic Polyphosphate in cardiac myocytes: The importance of polyP chain length for
318 energy metabolism and mPTP activation. Arch Biochem Biophys. 2019;662:177-89.
319 <http://doi.org/10.1016/j.febslet.2005.02.032>

320 10. Shiba T. Inorganic Polyphosphate and its chain-length dependency in tissue regeneration including
321 bone remodeling and teeth whitening. Inorganic Polyphosphates in Eukaryotic Cells.
322 2016;139-158. http://doi.org/10.1007/978-3-319-41073-9_10

323 11. Lager K J, Brouk M J, Bradford B J, et al. Impact of Supplemental Phosphorus Source on
324 Phosphorus Utilization in Lactating Dairy Cattle. Rep Prog. 2009. <http://hdl.handle.net/2097/1404>

- 325 12. Elliott R P, Straka R P, Garibaldi J A. Polyphosphate inhibition of growth of pseudomonads from
326 poultry meat. Appl Microbiol. 1964;12(6):517-522.
327 <https://doi.org/10.1128/am.12.6.517-522.1964>
- 328 13. Froning GW. Effect of Polyphosphates on Binding Properties of Chicken Meat. Poult Sci.
329 1965;44(4):1104-7. <http://doi.org/10.3382/ps.0441104>
- 330 14. Kornegay E T. Supplementation of lysine, ammonium poly-phosphate and urea in diets for
331 growing-finishing pigs. J Anim Sci. 1972;34(1):55-63. <https://doi.org/10.2527/jas1972.34155x>
- 332 15. Moon SG, Kothari D, Kim WL, Lee WD, Kim KI, Kim JI, et al. Feasibility of sodium long chain
333 Polyphosphate as a potential growth promoter in broilers. J Anim Sci Technol.
334 2021;63(6):1286-300. <http://doi.org/10.5187/jast.2021.e110>
- 335 16. Niu KM, Khosravi S, Kothari D, Lee WD, Lim JM, Lee BJ, et al. Effects of dietary multi-strain
336 probiotics supplementation in a low fishmeal diet on growth performance, nutrient utilization,
337 proximate composition, immune parameters, and gut microbiota of juvenile olive flounder
338 (*Paralichthys olivaceus*). Fish Shellfish Immunol. 2019;93:258-68.
339 <https://doi.org/10.1016/j.fsi.2019.07.056>
- 340 17. Lebrini M, Bentiss F, Chihib N-E, Jama C, Hornez JP, Lagrenée M. Polyphosphate derivatives of
341 guanidine and urea copolymer: Inhibiting corrosion effect of Armco iron in acid solution and
342 antibacterial activity. Corros. Sci. 2008;50(10):2914-8.
343 <https://doi.org/10.1016/j.corsci.2008.07.003>
- 344 18. Lorencova E, Vltavska P, Budinsky P, Koutny M. Antibacterial effect of phosphates and
345 Polyphosphates with different chain length. J Environ Sci Health A Tox Hazard Subst Environ Eng.
346 2012;47(14):2241-5. <https://doi.org/10.1080/10934529.2012.707544>
- 347 19. Maier S K, Scherer S, Loessner M J. Long-chain Polyphosphate causes cell lysis and inhibits
348 *Bacillus Cereus* septum formation, which is dependent on divalent cations. Appl Environ.
349 Microbiol. 1999;65(9):3942-3949. <https://doi.org/10.1128/AEM.65.9.3942-3949.1999>
- 350 20. Lee RM, Hartman PA, Stahr HM, Olson DG, Williams FD. Antibacterial Mechanism of
351 Long-Chain Polyphosphates in *Staphylococcus aureus*. J Food Prot. 1994;57(4):289-94.
352 <https://doi.org/10.4315/0362-028X-57.4.289>
- 353 21. Buňková L, Pleva P, Buňka F, Valášek P, Kráčmar S. Antibacterial effects of commercially
354 available phosphates on selected microorganisms. Acta Univ Agric Silvic Mendelianae Brun.
355 2014;56(5):19-24.
- 356 22. Fusieger A, da Silva R R, de Jesus Silva S R, et al. Inhibitory activity of an emulsifying salt
357 Polyphosphates (JOHA HBS®) used in processed cheese: An in vitro analysis of its antibacterial
358 potential. LWT, 2022;167:113777. <https://doi.org/10.1016/j.lwt.2022.113777>

- 359 23. Zaika LL, Kim AH. Effect of Sodium Polyphosphates on Growth of *Listeria monocytogenes*. J
360 Food Prot. 1993;56(7):577-80. <https://doi.org/10.4315/0362-028X-56.7.577>
- 361 24. Qureshi A A, Burger W C, Prentice N, et al. Regulation of lipid metabolism in chicken liver by
362 dietary cereals. J Nutr. 1980;110(3):388-393. <https://doi.org/10.1093/jn/110.3.388>
- 363 25. Weiner M L, Salminen W F, Larson P R, et al. Toxicological review of inorganic phosphates.
364 Food Chem Toxicol. 2001;39(8):759-786. [https://doi.org/10.1016/S0278-6915\(01\)00028-X](https://doi.org/10.1016/S0278-6915(01)00028-X)
- 365 26. Hong S H, Park S J, Lee S, et al. Biological effects of inorganic phosphate: potential signal of
366 toxicity. The Journal of toxicological sciences. 2015;40(1):55-69.
367 <https://doi.org/10.2131/jts.40.55>
- 368 27. Wang X, Farnell YZ, Peebles ED, Kiess AS, Wamsley KG, Zhai W. Effects of prebiotics,
369 probiotics, and their combination on growth performance, small intestine morphology, and
370 resident *Lactobacillus* of male broilers. Poult Sci. 2016;95(6):1332-40.
371 <http://doi.org/10.3382/ps/pew030>
- 372 28. Vieira V, Marx FO, Bassi LS, Santos MC, Oba A, de Oliveira SG, et al. Effect of age and
373 different doses of dietary vitamin E on breast meat qualitative characteristics of finishing broilers.
374 Anim Nutr. 2021;7(1):163-7. <http://doi.org/10.1016/j.aninu.2020.08.004>
- 375 29. Watts B M. Polyphosphates as synergistic antioxidants. J Am Oil Chem Soc. 1950;27(2):48-51.
376 <https://doi.org/10.1007/BF02634877>
- 377 30. Trout GR. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat
378 as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. J
379 Food Sci. 1989;54(3):536-540. <https://doi.org/10.1111/j.1365-2621.1989.tb04644.x>
- 380 31. Han X, Ye H. Overview of Lipidomic Analysis of Triglyceride Molecular Species in Biological
381 Lipid Extracts. J Agric Food Chem. 2021;69(32):8895-909. <http://doi.org/10.1093/ps/86.4.654>
- 382 32. Zhou Y, Chen Z, Lin Q, Yang Y, Hang Y, Zhou X, et al. Nuciferine reduced fat deposition by
383 controlling triglyceride and cholesterol concentration in broiler chickens. Poult Sci.
384 2020;99(12):7101-8. <http://doi.org/10.1016/j.psj.2020.09.013>
- 385 33. Ito T, Yamamoto S, Yamaguchi K, Sato M, Kaneko Y, Goto S, et al. Inorganic Polyphosphate
386 potentiates lipopolysaccharide-induced macrophage inflammatory response. J Biol Chem.
387 2020;295(12):4014-23. <https://doi.org/10.1074/jbc.RA119.011763>
- 388 34. Bowlin MQ, Gray MJ. Inorganic Polyphosphate in host and microbe biology. Trends Microbiol.
389 2021;29(11):1013-23. <https://doi.org/10.1016/j.tim.2021.02.002>
- 390 35. Garcia-Gonzalez MA, Lanas A, Santolaria S, Crusius JB, Serrano MT, Pena AS. The

391 polymorphic IL-1B and IL-1RN genes in the aetiopathogenesis of peptic ulcer. Clin Exp
392 Immunol. 2001;125(3):368-75. <https://doi.org/10.1046/j.1365-2249.2001.01593.x>

393 36. De Vries MC, Vaughan EE, Kleerebezem M, de Vos WM. *Lactobacillus plantarum*—survival,
394 functional and potential probiotic properties in the human intestinal tract. Int Dairy J.
395 2006;16(9):1018-28. <http://doi.org/10.1016/j.idairyj.2005.09.003>

396 37. Saiki A, Ishida Y, Segawa S, Hirota R, Nakamura T, Kuroda A. A *Lactobacillus* mutant capable
397 of accumulating long-chain Polyphosphates that enhance intestinal barrier function. Biosci
398 Biotechnol Biochem. 2016;80(5):955-61. <http://doi.org/10.1080/09168451.2015.1135041>

399 38. Safaei H G, Jalali M, Hosseini A, et al. The prevalence of bacterial contamination of table eggs
400 from retails markets by *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni* and
401 *Escherichia coli* in Shahrekord, Iran. Jundishapur J Microbiol. 2011;4(4).

402 39. Mani S, Wierzba T, Walker RI. Status of vaccine research and development for *Shigella*. Vaccine.
403 2016;34(26):2887-94. <http://doi.org/10.1016/j.vaccine.2016.02.075>

404 40. Xie L, Chen T, Qi X, et al. Exopolysaccharides from Genistein-Stimulated *Monascus purpureus*
405 Ameliorate Cyclophosphamide-Induced Intestinal Injury via PI3K/AKT-MAPKs/NF-kappaB
406 Pathways and Regulation of Gut Microbiota. J Agric Food Chem. 2023;71(35):12986-3002.
407 <https://doi.org/10.1021/acs.jafc.3c03186>

408 41. Zafar H, Saier Jr M H. Gut Bacteroides species in health and disease. Gut microbes.
409 2021;13(1):1848158. <https://doi.org/10.1080/19490976.2020.1848158>

410 42. Jin S, Chen P, Yang J, et al. *Phocaeicola vulgatus* alleviates diet-induced metabolic
411 dysfunction-associated steatotic liver disease progression by downregulating histone acetylation
412 level via 3-HPAA. Gut Microbes. 2024;16(1):2309683.
413 <https://doi.org/10.1080/19490976.2024.2309683>

414 43. Miquel S, Martin R, Rossi O, et al. *Faecalibacterium prausnitzii* and human intestinal health.
415 Current opinion in microbiology. 2013;16(3):255-261. <https://doi.org/10.1016/j.mib.2013.06.003>

416 44. Daillère R, Vétizou M, Waldschmitt N, et al. *Enterococcus hirae* and *Barnesiella intestinihominis*
417 facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. Immunity.
418 2016;45(4):931-943.

419

420

421 **Tables and Figures**422 **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
NaHCO ₃	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

423 ^aPer kilogram of diet, mineral premix contained the following: 80 mg Fe; 50 mg Zn; 60 mg Mn; 0.3 mg Co; 10 mg Cu;
424 0.2 mg Se.

425 ^bPer kilogram of diet, vitamin premix ingredients were as follows: vitamin A, 80,000 IU; vitamin D₃, 1600 IU; vitamin E,
426 20 IU; vitamin K₃, 8 mg; vitamin B₁, 8 mg; vitamin B₂, 24 mg; vitamin B₆, 12 mg; vitamin B₁₂, 0.040 mg; pantothenic
427 acid, 40 mg; folic acid, 4 mg; nicotinic acid, 120 mg. Met, formethionine; Cys, cysteine; and AMEn, apparent
428 metabolizable energy.

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

Gene ¹⁾	Forward primer	Reverse primer
β -actin	ACCAACTGGGACGACATGGA	GTGATGACCTGGCCGTCAG
IL-1 β	AGAGATGGCGTTCGTTCCC	GCAGTCAGCGCCCACTTA
IL-1RN	ATTGGGGCATCTCATGGGTG	GCTCAGCACAGCTGGAAGTA
IL-6	AGAAGCCGCACCATGAACTT	TGGTAACAGAGGATTGTGCC
TNF α	ATGACCACGCTCTTTCCGT	TTAATCCACTCCCACCACCC

430 ¹⁾ β -actin, Beta-actin; IL-1 β , Interleukin-1 beta; IL-1RN, Interleukin 1 receptor antagonist; IL-6, Interleukin 6; TNF α , 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
<i>Listeria monocytogenes</i>	ND ²⁾	ND	ND
<i>Shigella sonnei</i>	ND	20.76±0.09	15.99±0.62
<i>Salmonella enterica</i> ser. Gallinarum	ND	ND	ND
<i>Klebsiella pneumoniae</i>	ND	ND	ND
<i>Pseudomonas aeruginosa</i>	ND	11.00±0.65	9.89±0.74
<i>Salmonella enterica</i> ser. Pullorum	ND	13.78±0.15	10.20±0.20
<i>Escherichia coli</i> 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⁾	<i>p</i> -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.

442

443 **Table 5.** Effect of different length Polyphosphates for meat quality

Item ²⁾	Treatment ¹⁾				SEM ³⁾	<i>p-value</i>
	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

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

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

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

448

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

Item(logCFU/g)	Treatment ¹⁾				SEM ²⁾	<i>p-value</i>
	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⁾	<i>p</i> -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.

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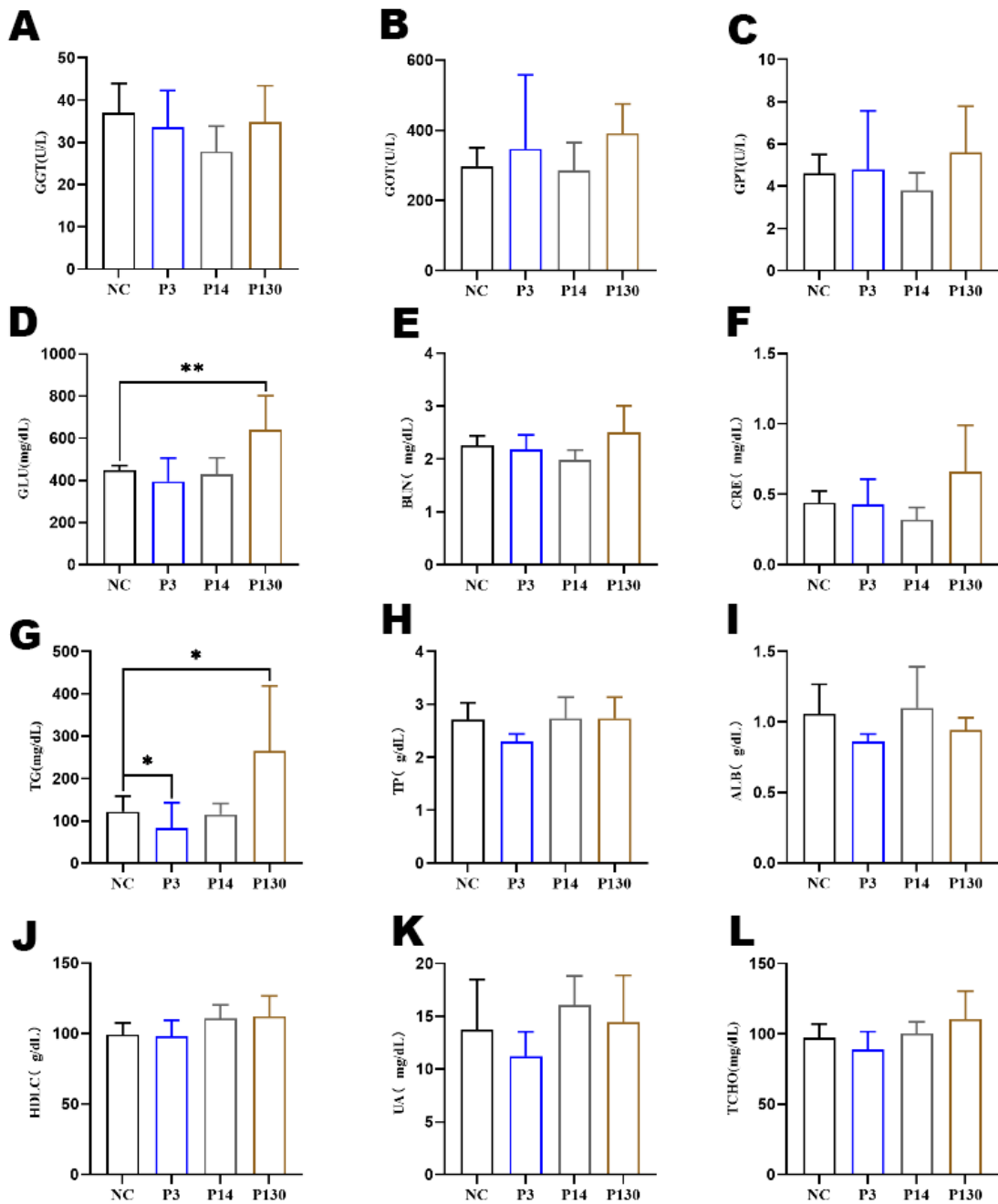
Table 8. Effect of different chain length polyphosphates on phylum and genus in the intestinal flora

Item	Treatment ¹⁾				SEM ²⁾	<i>p</i> -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
Pseudesccherichia	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.

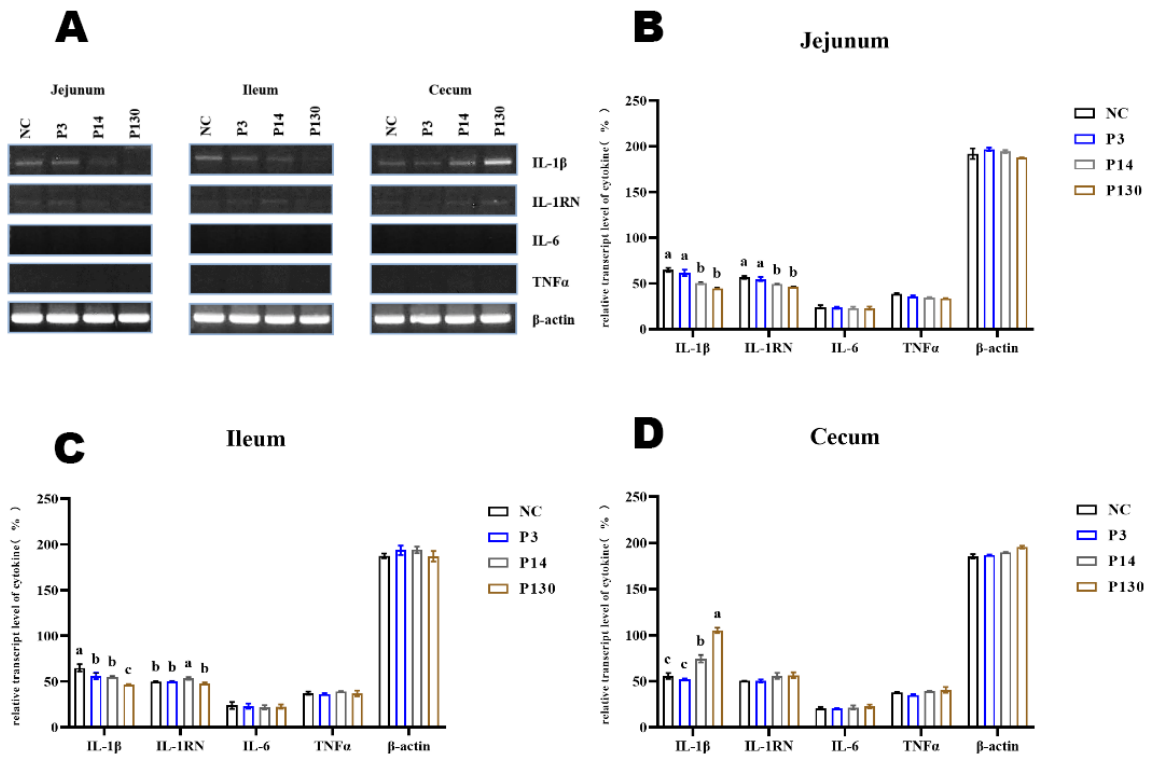
465 ²⁾Values are presented as mean and standard error of mean (SEM).

466 ^{a-b)}in the same row, with significant differences indicated between values marked differently above (*p*
467 ≤ 0.05).



469

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



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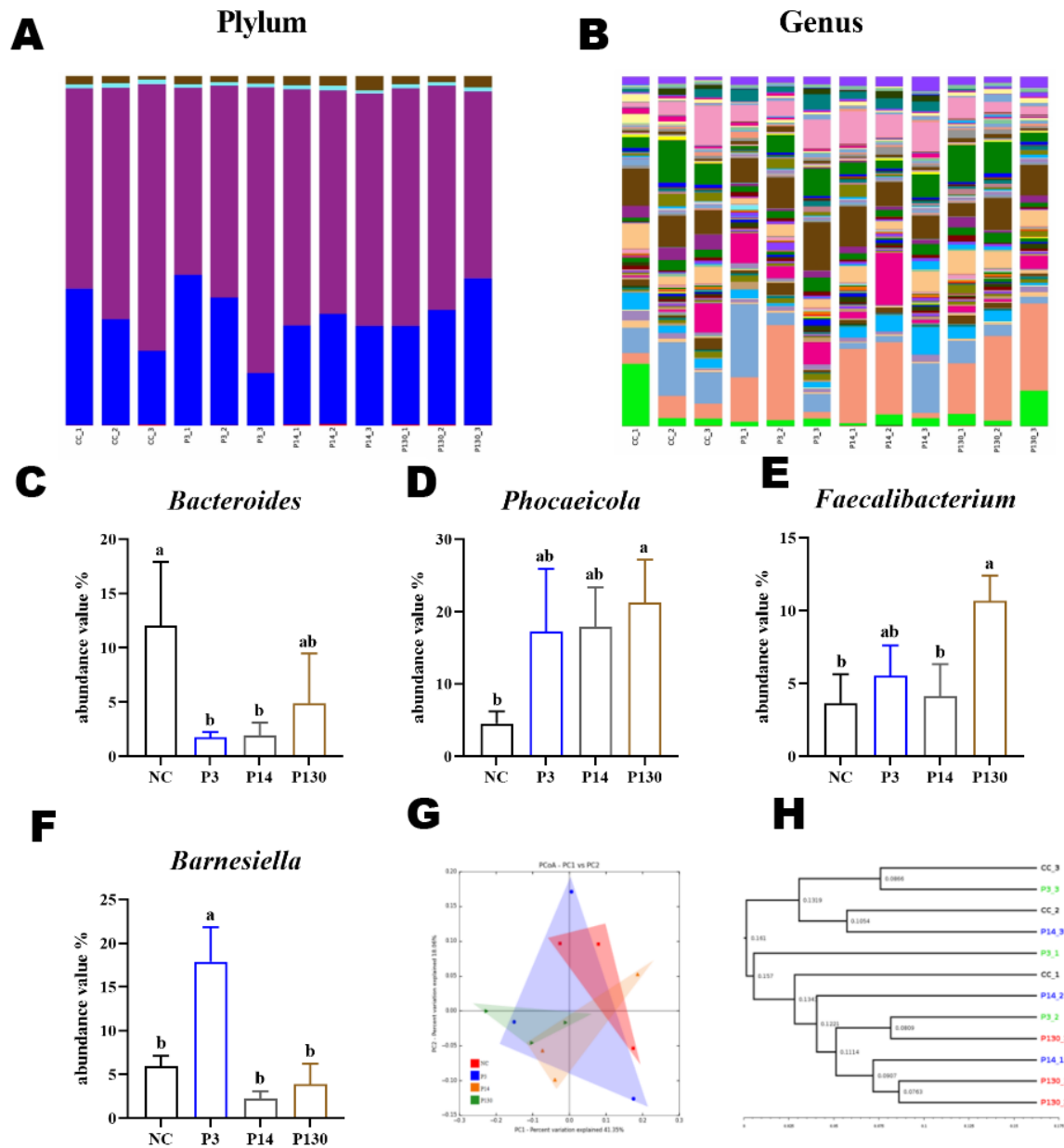
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Fig. 2. Expression of four different avian pro-inflammatory cytokine genes (a), interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1RN), IL-6, and tumor necrosis factor (TNF-α), in three intestinal organs, jejunum (b), ileum (c), and cecum(d) after 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 (MCP), P130 = long chain polyphosphates (LCPP). ^{a-d} mean within a column within a main effect are significantly different ($p < 0.05$)



483

484 **Fig. 3. Effect of different length polyphosphates for intestinal flora in cecum.** (a) Phylum level. (b-f)
 485 Relative abundances of the gut microbiota at the genus level. (g-h) PCoA analysis based on Bray-Curtis
 486 distance and Phylogenetic tree of the cecum microbiota, where the confidence interval is 95%.
 487 ^{a-d} mean within a column within a main effect are significantly different ($p < 0.05$, $n = 3$).
 488