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Replacing soybean meal with sunflower meal in laying hens rations and its effects on cecal volatile fatty acids profile and intestinal microbial colonization

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ABSTRACT: This experiment was performed to evaluate the effects of replacing different levels of soybean meal with sunflower meal (with and without enzyme) on cecal volatile fatty acid profile and intestinal microbial colonization in laying hens. In this experiment, 360 laying hens (1530 ± 20 g) from the age of 47 to 57 weeks, in 9 treatments and five replications (8 hens in each replicate) were performed as a 3×3 factorial experiment in a completely randomized design for ten weeks. The results showed that the replacement of 30 and 60 percent sunflower meal and enzyme (100 and 200 gr/ton) has significantly improved the concentration of n-valeric acid on the cecum of laying hens ($P < 0.05$). Simultaneously, increasing 30 percentage of sunflower meal or 100 of an enzyme enhanced isovaleric acid, n-butyric acid, isobutyric acid, propionic acid, and acetic acid ($P < 0.05$). Reduction in intestinal log *E. coli* was discovered for samples treated with 30 and 60 percent of sunflower ($P < 0.05$), but for log baglus that treated with 30 and 60 percent of sunflower showed higher ($P < 0.05$). The main effects of the enzyme had no significant impact on the cecal microbial population ($P > 0.05$) while, the effect of meal source and enzyme additive interactions on the tested parameters was significant ($P < 0.05$). Hence, sunflower meal could be used as an alternative protein source in laying hens ration to improve cecal VFA profile and reduce cecal *E. coli* population. Substitution of sunflower meal in laying hens is recommended.

Keywords: Cecal VFA profile, Laying hens, Microbial population, Soybean meal, Sunflower meal

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INTRODUCTION

The use of sunflower meal in poultry diets can be beneficial because of the effect of fiber on lowering cholesterol and the complication of cannibalism (Baghban-Kanani et al., 2019). According to Shi et al. (2012), there are two hypotheses to explain that sunflower meal lowers plasma cholesterol and egg yolk: 1) Dietary fiber indirectly inhibits cholesterol synthesis 2) Dietary fiber increases the secretion of cholesterol into the bile. Research has shown that the use of high-fiber feed (insoluble non-starch polysaccharides) in poultry diets can improve the production performance of poultry by increasing the digestibility of nutrients and the intestinal microbial population (Walugembe et al., 2015). Dietary crude fiber provides the energy needed by gastrointestinal bacteria, especially the colon, and facilitates their growth. This increase in bacterial growth can improve the digestibility of crude fiber in the diet. The high metabolism of bacteria leads to increased production of volatile fatty acids such as lactic acid, butyric acid, and propionic acid at the end of the digestive system, which can lower the pH of the intestine and even the gills and increase the villi of the small intestine and consequently increase the level of intestinal absorption (Scholz-Ahrens et al., 2007; González-Alvarado et al., 2008; Jiang et al., 2017; Nogal et al., 2021). Research has shown that low pH in the end parts of the digestion system increases the bioavailability of minerals, especially calcium, zinc, and magnesium (Philipp Schuchardt and Hahn, 2017; Corte-Real and Bohn, 2018; Gibson et al., 2018; Rogaska et al., 2018). It has also been shown that this decrease in pH can inhibit the growth of protons in the cell membrane of the harmful microbial flora of the gastrointestinal tract, such as *E. coli* and *Salmonella* (Aprisal and Husmaini, 2020; Roy et al., 2021), by destroying their motility. As a result, it improves intestinal health in poultry (Alloui et al., 2013). Secondary structures negatively affect poultry performance, as intestinal digestion decreases and high intestinal viscosity adversely affects the gut's immune response and microbial proliferation. To solve this problem, some exogenous enzymes such as protease, phytase, avizyme, β -mannanase, and β -glucanase could be added to poultry diets to help in the digestion of fibers and to decline their negative impact on poultry productivity and health (Saeed et al., 2019). Hence, we hypothesized that replacing sunflower meal in the diet of laying will improve gastrointestinal health and calcium absorption. Additional investigation of cecal volatile

fatty acids profile would further our understanding on development of the cecal microflora. Therefore, this study aimed to evaluate the replacement of different levels of soybean meal with sunflower meal (with and without enzyme) on cecal volatile fatty acids profile and intestinal microbial colonization in laying hens.

MATERIAL AND METHODS

The animal welfare committee of Islamic Azad University (Maragheh Branch) approved the animal care protocol used in this experiment (no. 1319-IAU. 01.25.2017). A total of 360 laying hens (1530 ± 20 g) from the age of 47 to 57 weeks in 9 treatments and 5 replications (8 hens in each replicate) were divided into treatments in a 3×3 factorial arrangement consisting of sunflower meal (0, 30, and 60) and enzyme additive (0, 100 and 200 gr/ton) in a completely randomized design for ten weeks. The hens had access to *ad libitum* feed and water, and the lighting program was 16 hours of light and 8 hours of darkness. The hens were fed a practical ration, adjusted to the recommended nutrient requirements of the 36 Hy-Line strains (Management Guide, 2020). To examine the cecal microbial population at the end of the experimental period, two birds from each replicate (close to the average body weight) were randomly selected and anesthetized using sodium thiopental. Samples were taken from the cecum, transferred to the laboratory in containers containing ice. Conventional culture medium was used to count *Lactobacillus* bacteria at 37 °C for 48 hours under anaerobic conditions and count *Escherichia coli* bacteria at 37 °C for 24 hours under aerobic conditions (Pang and Applegate, 2007). Cecal VFAs were determined with gas chromatography (Agilent 7890A, Agilent Technologies, Santa Clara, CA) following Shen et al. (2009).

The data were analyzed using the general linear model procedure of SAS (SAS Institute, Inc., Cary, North Carolina, USA). Data were log-transformed before analyzing in case of unequal variances (Palangi, 2021). The linear model was:

$$Y_{ijk} = \mu + A_i + B_j + AC_{ij} + BC_{jk} + \varepsilon_{ijkl}$$

where: Y_{ijkl} = a dependent variable, μ = overall mean, A_i = the effect of sunflower meal replacement, B_j = the effect of Multi-enzyme, AB_{ij} = the interaction of factors A and B, and ε_{ijkl} = the residual deviation of the observation from the effects in the model. Tukey's test at the 5% level of probability was used to compare means.

RESULTS

Cecal microbial population

The effects of replacing soybean meal with sunflower meal (with and without enzyme) in laying hens rations on cecal microbial population are shown in Table 1. As can be seen in Table 1, the use of sunflower meal was affected the *Bacillus* and *E. coli* population ($P < 0.05$). So that increasing the sunflower meal leads to increasing the *Bacillus* and reducing the *E. coli* population.

The effects of sunflower meal \times multi-enzyme, on *Bacillus* and *E. coli* population in all replacement ratios were significant ($P < 0.05$). Interestingly, the main effects of the enzyme on the measured parameters were not significant ($P > 0.05$). While the 0% sunflower meal \times 200 gr/ton multi-enzyme interactions showed the highest *Bacillus* population, the highest *E. coli* population were observed for 0% sunflower meal \times 0 gr/ton multi-enzyme interactions.

Cecal volatile fatty acids

The effects of replacing soybean meal with sunflower meal in laying hens rations on cecal volatile fatty acids are shown in Table 2. Cecal VFA composition significantly improved using sunflower meal ($P < 0.05$), the 30% replacement level had the largest increase in the composition of VFA profiles. The addition of 100 gr/ton enzyme also had the greatest effect on the VFA profile ($P < 0.05$). Interactions of using 30% sunflower pulp with 100 gr/ton of the enzyme had the highest amount of VFA.

DISCUSSION

Cecal microbial population

Various experiments have shown that diet composition (Feng et al., 2020; Guo et al., 2020), type and amount of NSP (Yang et al., 2020; Farahat et al., 2021), intestinal viscosity (Sjofjan et al., 2019), grain type (Wu et al., 2017), antibiotics (Levesque et al., 2017; Cai et al., 2018), probiotics (Qorbanpour et al., 2018; Xu et al., 2019), prebiotics (Teng and Kim,

2018), organic acids (Yang et al., 2019), and other diet-related factors affect the intestinal microbial population. Insoluble polysaccharides affect the intestinal microflora and reduce the incidence of intestinal problems such as enteritis (Zhou et al., 2018; Ge et al., 2021; Tejeda and Kim, 2021), but the beneficial effects of these compounds on the small intestinal microflora and their mechanism are not exactly known. Various factors such as host, interactions of microorganisms, and feed affect the microbial population of the cecum (Kers et al., 2018). Unabsorbed nutrients at the end of the small intestine are potential substrates for microbes in the cecum of the gastrointestinal tract. Dietary changes affect the accessibility of the substrate and the composition of the bacterial species at the end of the gastrointestinal tract. Sunflower meal increases intestinal health by acidifying the upper parts of the gastrointestinal tract and leads to a natural barrier against pathogenic bacteria sensitive to an acidic environment, including *E. coli*. *Lactobacillus* are considered beneficial microorganisms due to their ability to inhibit the growth of pathogenic bacteria (Shokryazdan et al., 2017; Alizadeh et al., 2020). Therefore, increasing the population of these bacteria due to high levels of sunflower meal is also one of the beneficial effects of this protein source in laying hens. The main reasons for an increase in useful intestinal bacteria such as *Bacillus* may be related to the amount of dietary fiber as the amount of fiber in sunflower meals in contrast to soybean meal is high, so the production of bacteria like *Bacillus* be more. This kind of bacteria can improve the intestinal microbial population. Some research shows the beneficial effects of intestinal microbial composition on health and performance (Roberts et al., 2015; Tarabees et al., 2020; Alwaleed et al., 2021).

Cecal volatile fatty acids

Short-chain fatty acids, such as acetate, butyrate, and propionate, are produced during carbohydrate fermentation, while branched-chain fatty acids are a product of protein fermentation in the gastrointestinal tract (Ndazigaruye et al., 2019). Changes in microbial

Table 1. The effect of replacing soybean meal with sunflower meal (with and without enzyme) in laying hens rations on cecal microbial population

Trt	Sunflower meal					Multi-enzyme					Interaction of sunflower meal and multi-enzyme										
	0	30	60	SEM	P value	0	100	200	SEM	P value	0×0	0×100	0×200	30×0	30×100	30×200	60×0	60×100	60×200	SEM	P value
Bacillus	5.76 ^b	6.39 ^a	6.22 ^{ab}	0.864	0.0257	6.09	5.76	6.50	0.1624	0.0107	5.20 ^b	5.03 ^b	7.04 ^a	6.61 ^a	5.91 ^{ab}	6.65 ^a	6.48 ^{ab}	6.36 ^{ab}	5.82 ^{ab}	0.2814	0.0003
Ecoli	6.18 ^a	5.25 ^b	5.38 ^b	0.1450	<0.0001	5.75	5.39	5.68	0.1250	0.1132	6.92 ^a	5.45 ^b	6.09 ^{ab}	5.19 ^b	5.21 ^b	5.34 ^b	5.13 ^b	5.40 ^b	5.60 ^b	0.2166	0.0041

SEM: Standard Error Means

Table 2. The effect of replacing soybean meal with sunflower meal (with and without enzyme) in laying hens rations on cecal VFA composition

TRT		nVA	iVA	nBA	iBA	PA	AA
Sunflower							
0		0.381 ^b	1.013 ^b	3.595	1.067 ^b	7.659	6.382 ^c
30		0.786 ^a	1.409 ^a	4.225	2.735 ^a	7.329	25.390 ^a
60		0.710 ^a	0.977 ^b	3.383	0.983 ^b	7.733	15.009 ^b
SEM		0.1150	0.0269	0.3586	0.0544	0.1798	0.4469
P Value		0.0400	<0.0001	0.2386	<0.0001	0.2521	<0.0001
Enzyme							
0		0.375 ^b	0.979 ^b	3.084 ^b	0.997 ^b	7.643 ^b	8.799 ^c
100		1.029 ^a	1.573 ^a	5.125 ^a	3.107 ^a	9.576 ^a	26.416 ^a
200		0.274 ^b	0.847 ^c	2.989 ^b	0.681 ^c	5.503 ^c	11.486 ^b
SEM		0.1149	0.0269	0.3586	0.0544	0.1798	0.4469
P Value		<0.0005	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
Sunflower × Enzyme							
0	0	0.526 ^b	1.246 ^b	2.233 ^{bc}	1.276 ^b	7.146 ^d	9.616 ^c
0	100	0.000 ^c	0.736 ^{ef}	2.976 ^{bc}	0.898 ^{bc}	6.716 ^{de}	3.666 ^e
0	200	0.616 ^b	1.056 ^{bed}	4.576 ^b	1.026 ^{bc}	9.116 ^c	5.866 ^{de}
30	0	0.000 ^c	0.616 ^{fg}	1.754 ^{bc}	0.698 ^c	3.206 ^g	11.346 ^c
30	100	2.360 ^a	3.126 ^a	9.882 ^a	7.506 ^a	17.046 ^a	56.106 ^a
30	200	0.000 ^c	0.486 ^g	1.036 ^c	0.000 ^d	1.736 ^h	8.476 ^{cd}
60	0	0.600 ^b	1.076 ^{bc}	4.266 ^b	1.016 ^{bc}	12.576 ^b	5.436 ^{de}
60	100	0.726 ^b	0.856 ^{de}	2.256 ^{bc}	0.916 ^{bc}	4.966 ^f	19.476 ^b
60	200	0.806 ^b	1.000 ^{ed}	3.356 ^{bc}	1.016 ^{bc}	5.656 ^{ef}	20.116 ^b
SEM		0.1992	0.0467	0.6211	0.0942	0.3114	0.7742
P Value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

nVA: n-valeric acid, iVA: isovaleric acid, nBA: n-butyric acid, iBA: isobutyric acid, PA: propionic acid, and AA: acetic acid; TRT: Treatments SEM: Standard Error Means

flora reflect changes in VFA composition. Gram-positive organisms were assumed to be involved in fermenting polysaccharides into VFA, especially acetate and butyrate (Seon et al., 2014). VFA is responsible for reducing *E. coli*, and significant negative correlations were observed between the numbers of Enterobacteriaceae (*E. coli*) and acetate in the ceca (Zhu et al., 2015). VFAs may play a key role in developing microflora in the ceca of laying hens. Still, it is not well understood the relationship and the mechanisms of reducing *E. coli* cecal colonization and cecal VFA. Furthermore, van der Wielen et al. (2000) investigated that the increased concentrations of butyric acid were related to decreased amounts of *E. coli*. Therefore, the high concentration of butyric acid produced in the ceca of hens may play an important role in the mechanism that inhibits *E. coli* population. Fibers are the main source for fermentation and production of new substances such as volatile fatty acids. Fibers migrate to the large intestine and are fermented. One of the main products in this region is volatile fatty acids.

These acids have some advantages, such as improving intestinal pH and decreasing it. Reducing intestinal pH has different advantages with health, immune, and production (Sun et al., 2020; Kim et al., 2020).

CONCLUSION

In conclusion, supplementation of sunflower meal in laying hens diets can stimulate the activity of beneficial cecal microbial population and result in increased production of VFA. The results indicate that sunflower meal has the potential to improve immune status of laying hens.

CONFLICT OF INTEREST

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

ETHICS APPROVAL

Protocol (no. 1319-IAU. 01.25.2017) was ap-

proved by the experimental animal ethics committee of Maragheh Islamic Azad University.

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REFERENCES

- Alizadeh M, Shojadoost B, Astill J, Taha-Abdelaziz K, Karimi SH, Bavanthasivam J, Sharif S (2020) Effects of in ovo inoculation of multi-strain lactobacilli on cytokine gene expression and antibody-mediated Immune responses in chickens. *Frontiers Vet Sci* 7(February): 105. <https://doi.org/10.3389/fvets.2020.00105>
- Alloui MN, Szczurek W, Świątkiewicz S (2013) The usefulness of pre-biotics and probiotics in modern poultry nutrition: a review/. *Annals Anim Sci* 13 (1): 17-32.
- Alwaleed EA, El-Sheekh M, Abdel-Daim MM, Saber H (2021) Effects of spirulina platensis and Amphora coffeaeformis as dietary supplements on blood biochemical parameters, intestinal microbial population, and productive performance in broiler chickens. *Environ Sci Pollut Res* 28(2): 1801-1811.
- Aprisal YM, Husmaini H (2020) Selection of lactic acid bacteria (LAB) origin of food fermentation probiotic mixed as candidate for broiler. *Int J Environ Agric Biotechnol* 5: 68-75.
- Baghban-Kanani P, Janmohammadi H, Ostadrahimi AR (2019) Effect of different levels of sunflower meal and niacin on performance, biochemical parameters, antioxidant status, and egg yolk cholesterol of laying hens. *Iranian J Applied Anim Sci* 9(4): 737-746.
- Cai M, Ma S, Hu R, Tomberlin JK, Thomashow LS, Zheng L, Zhang J (2018) Rapidly mitigating antibiotic resistant risks in chicken manure by *Hermetia illucens* bioconversion with intestinal microflora. *Environ Microbiol* 20(11): 4051-4062.
- Corte-Real J, Bohn T (2018) Interaction of divalent minerals with liposoluble nutrients and phytochemicals during digestion and influences on their bioavailability-a review. *Food Chem* 252(June): 285-293. <https://doi.org/10.1016/j.foodchem.2018.01.113>
- Farahat M, Ibrahim D, Kishawy ATY, Abdallah HM, Hernandez-Santana A, Attia G (2021) Effect of cereal type and plant extract addition on the growth performance, intestinal morphology, caecal microflora, and gut barriers gene expression of broiler chickens. *Animal* 15(3): 100056.
- Feng Y, Wang L, Khan A, Zhao R, Wei S, Jing X (2020) Fermented wheat bran by xylanase-producing *Bacillus cereus* boosts the intestinal microflora of broiler chickens. *Poult Sci* 99(1): 263-271.
- Ge Y, Ahmed S, Yao W, You L, Zheng J, Hileuskaya K (2021) Regulation effects of indigestible dietary polysaccharides on intestinal microflora: An overview. *J Food Biochem* 45(1): e13564.
- Gibson RS, Raboy V, King JC (2018) Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nut Rev* 76(11): 793-804.
- González-Alvarado JM, Jiménez-Moreno E, Valencia DG, Lázaro R, Mateos GG (2008) Effects of fiber source and heat processing of the cereal on the development and pH of the gastrointestinal tract of broilers fed diets based on corn or rice. *Poult Sci* 87 (9): 1779-1795.
- Guo WL, Deng JC, Pan YY, Xu JX, Hong JL, Shi FF, Lv XC (2020) Hypoglycemic and hypolipidemic activities of *Grifola frondosa* polysaccharides and their relationships with the modulation of intestinal microflora in diabetic mice induced by high-fat diet and streptozotocin. *Int J Biological Macromol* 153(June): 1231-1240. <https://doi.org/10.1016/j.ijbiomac.2019.10.253>
- Palangi, V. (2021). In situ ruminal degradation of sawtooth tree leaves using different mathematical models. *Revista MVZ Córdoba*, 26(3).
- J Tejada O, K Kim W (2021) Role of dietary fiber in poultry nutrition. *Animals* 11(2): 461.
- Jiang H, Che D, Qin G, Kong X, Hamdy Farouk M (2017) Effects of dietary non-fiber carbohydrates on composition and function of gut microbiome in monogastrics: A review. *Protein Pept Lett* 24(5): 432-441.
- Kers JG, Velkers FC, Fischer EA, Hermes GD, Stegeman JA, Smidt H (2018) Host and environmental factors affecting the intestinal microbiota in chickens. *Frontiers Microbiol* 9(February): 235. <https://doi.org/10.3389/fmicb.2018.00235>
- Kim, D. H., Lee, Y. K., Lee, S. D., Kim, S. H., Lee, S. R., Lee, H. G., & Lee, K. W. (2020). Changes in production parameters, egg qualities, fecal volatile fatty acids, nutrient digestibility, and plasma parameters in laying hens exposed to ambient temperature. *Frontiers in Veterinary Science*, 7, 412. <https://doi.org/10.3389/fvets.2020.00412>
- Levesque CL, Turner J, Li J, Wizzard P, St Pierre B, Lim D, Wales P (2017) In a neonatal piglet model of intestinal failure, administration of antibiotics and lack of enteral nutrition have a greater impact on intestinal microflora than surgical resection alone. *J Parenteral Enteral Nut* 41(6): 938-945.
- Management Guide - HY-LINE NORTH AMERICA (2020). <https://hylinena.com/wp-content/uploads/2020/02/W-36-English.pdf>
- Ndazigaruye G, Kim DH, Kang CW, Kang KR, Joo YJ, Lee SR, Lee KW (2019) Effects of low-protein diets and exogenous protease on growth performance, carcass traits, intestinal morphology, cecal volatile fatty acids and serum parameters in broilers. *Animals* 9(5): 226.
- Nogal A, Valdes AM, Menni C (2021) The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes* 13(1): 1-24.
- Pang Y, Applegate TJ (2007) Effects of dietary copper supplementation and copper source on digesta pH, calcium, zinc and copper complex size in the gastrointestinal tract of the broiler chicken. *Poult Sci* 86(3): 531-537. <https://doi.org/10.1093/ps/86.3.531>
- Philipp Schuchardt J, Hahn A (2017) Intestinal absorption and factors influencing bioavailability of magnesium-an update. *Current Nut Food Sci* 13(4): 260-278.
- Qorbanpour M, Fahim T, Javandel F, Nosrati M, Paz E, Seidavi A, Tufarelli V (2018) Effect of dietary ginger (*Zingiber officinale* Roscoe) and multi-strain probiotic on growth and carcass traits, blood biochemistry, immune responses and intestinal microflora in broiler chickens. *Animals* 8(7): 117.
- Roberts T, Wilson J, Guthrie A, Cookson K, Vancraeynest D, Schaeffer J, Clark S (2015) New issues and science in broiler chicken intestinal health: intestinal microbial composition, shifts, and impacts. *World's Poult Sci J* 71(2): 259-270.
- Rogaska A, Reguła J, Suliburska J, Krejpcio Z (2021) Comparison of the in vitro bioavailability of selected minerals from gluten-free breads enriched with grains and synthetic organic and non-organic compounds. *Molecules* 26(7): 2085.
- Roy PK, Ha AJW, Mizan MFR, Hossain MI, Ashrafudoulla M, Tushik SH, Ha SD (2021) Effects of environmental conditions (temperature, pH, and glucose) on biofilm formation of salmonella enterica serotype kentucky and virulence gene expression. *Poult Sci* 100(7): 101209. <https://doi.org/10.1016/j.psj.2021.101209>
- Saeed M, Ayaşan T, Alagawany M, El-Hack MEA, Abdel-Latif MA, Patra AK (2019) The role of β -mannanase (Hemicell) in improving poultry productivity, health and environment. *Brazilian J Poult Sci* 21(3): 1-8. <https://doi.org/10.1590/1806-9061-2019-1001>
- Scholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Açil Y, Schrezenmeier J (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *J Nut* 137 (3): 838S-846S.
- Seon J, Lee T, Lee SC, Pham HD, Woo HC, Song M (2014) Bacterial community structure in maximum volatile fatty acids production from alginate in acidogenesis. *Bioresour Technol* 157(April): 22-

27. <https://doi.org/10.1016/j.biortech.2014.01.072>
- Shen YB, Piao XS, Kim SW, Wang L, Liu P, Yoon I, Zhen YG (2009) Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. *J Anim Sci* 87(8): 2614-2624.
- Shi SR, Lu J, Tong HB, Zou JM, Wang KH (2012) Effects of graded replacement of soybean meal by sunflower seed meal in laying hen diets on hen performance, egg quality, egg fatty acid composition, and cholesterol content. *J Applied Poult Res* 21 (2): 367-374.
- Shokryazdan P, Faseleh Jahromi M, Liang JB, Ramasamy K, Sieo CC, Ho YW (2017) Effects of a *Lactobacillus salivarius* mixture on performance, intestinal health and serum lipids of broiler chickens. *Plos One* 12(5): e0175959.
- Sjofjan O, Irfan HD, Halim MH, Teguh H (2019) Effect of addition garlic flour as feed additive in digesta viscosity, microflora, and intestinal characteristic of native chicken crossbred. In IOP Conference Series: Earth Environ Sci 287(1): 012021). IOP Publishing.
- Sun B, Hou L, Yang Y (2020) Effects of altered dietary fiber on the gut microbiota, short-chain fatty acids and cecum of chickens during different growth periods. Preprints, 2020020109. <https://doi.org/10.20944/preprints202002.0109.v1>
- Tarabees R, El-Sayed MS, Shehata AA, Diab MS (2020) Effects of the probiotic candidate *E. faecalis*-1, the poulvac *E. coli* vaccine, and their combination on growth performance, caecal microbial composition, immune response, and protection against *E. coli* O78 challenge in broiler chickens. *Probiot Antimicrob Prot* 12(3): 860-872.
- Teng PY, Kim WK (2018) Roles of prebiotics in intestinal ecosystem of broilers. *Frontiers Vet Sci* 5(October): 245. <https://doi.org/10.3389/fvets.2018.00245>
- van der Wielen PW, Biesterveld S, Notermans S, Hofstra H, Urlings BA, van Knapen F (2000) Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied Environ Microbiol* 66(6): 2536-2540.
- Walugembe M, Hsieh JCF, Koszewski NJ, Lamont SJ, Persia ME, Rothschild MF (2015) Effects of dietary fiber on cecal short-chain fatty acid and cecal microbiota of broiler and laying-hen chicks. *Poult Sci* 94 (10): 2351-2359.
- Wu D, Wu SB, Choct M, Swick RA (2017) Performance, intestinal microflora, and amino acid digestibility altered by exogenous enzymes in broilers fed wheat-or sorghum-based diets. *J Anim Sci* 95(2): 740-751.
- Xu Y, Tian Y, Cao Y, Li J, Guo H, Su Y, Zhang L (2019) Probiotic properties of *Lactobacillus paracasei* subsp. *paracasei* L1 and its growth performance-promotion in chicken by improving the intestinal microflora. *Frontiers Physiol* 10: 937.
- Yang LJ, Zeng XF, Qiao SY (2020) Research advances on non-starch polysaccharide in the regulation of intestinal microflora in pigs. *Biotechnol Bulletin* 36(2): 9.
- Yang X, Liu Y, Yan F, Yang C, Yang X (2019) Effects of encapsulated organic acids and essential oils on intestinal barrier, microbial count, and bacterial metabolites in broiler chickens. *Poult Sci* 98(7): 2858-2865.
- Zhou H, Guo W, Zhang T, Xu B, Zhang D, Teng Z, Gao Y (2018) Response of goose intestinal microflora to the source and level of dietary fiber. *Poult Sci* 97(6): 2086-2094.
- Zhu W, Li D, Wang J, Wu H, Xia X, Bi W, Zhang L (2015) Effects of polymannuronate on performance, antioxidant capacity, immune status, cecal microflora, and volatile fatty acids in broiler chickens. *Poult Sci* 94(3): 345-352.