ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2017.403.414

Research Article
Effect of Direct-fed Microbials on Intestinal Villus Height in Broiler Chickens: A Systematic Review and Meta-Analysis of Controlled Trials

[1]Chhaiden Heak, [1,2]Peerapol Sukon, [1]Saijai Kongpechr, [1]Bundit Tengjaroenkul and [1]Kanlaya Chuachan

[1]Faculty of Veterinary Medicine, Khon Kaen University, Thailand
[2]Research Group for Animal Health Technology, Khon Kaen University, Thailand

Abstract

Objective: This systematic review and meta-analysis was conducted to evaluate the overall effect of direct-fed microbials (DFM) or probiotics on intestinal villus height in broiler chickens. **Materials and Methods:** Relevant studies were collected by searching PubMed, SCOPUS, Poultry Science Journal and Google Scholar. Studies of randomized or non-randomized controlled trials were included using DFM in broiler chickens and reporting intestinal villus height (VH) (the primary outcome), crypt depth (CD) or villus height-to-crypt depth (VH/CD) ratio. The overall effect on intestinal VH, CD or VH/CD was evaluated using the standardized mean difference (SMD) with a random effects meta-analysis. Subgroup analysis for the VH was planned a priori for 6 characteristics (broiler breed, sex, DFM product, microbial species, duration of treatment and application route). In addition, study quality (using SYRCLE's risk of bias tool) and publication bias were evaluated. **Results:** A total of 25 studies were identified (with a total of 296 comparisons) that met the inclusion criteria. Supplementation of DFM for broiler chickens was associated with increased VH in the small intestine (SMD = 3.38, 95% confidence interval (CI) (2.69, 4.06), $p<0.001$, $I^2 = 92$, n = 113 comparisons) and with greater VH/CD ratio in the small intestine (SMD = 2.73, 95% CI (2.09, 3.36, $p<0.001$, $I^2 = 91$, n = 87 comparisons). In subgroup analysis for the VH, significant differences among subgroups were found in 5 characteristics (broiler breed, DFM product, microbial species, duration of treatment and application route). Most studies were presented with unclear risk of bias. Publication bias was also observed. **Conclusion:** Supplementation with DFM for broiler chickens was associated with increased intestinal villus height.

Key words: Direct-fed microbials, probiotics, broiler chickens, villus height, systematic review, meta-analysis

Citation: Chhaiden Heak, Peerapol Sukon, Saijai Kongpechr, Bundit Tengjaroenkul and Kanlaya Chuachan, 2017. Effect of direct-fed microbials on intestinal villus height in broiler chickens: a systematic review and meta-analysis of controlled trials. Int. J. Poult. Sci., 16: 403-414.

Corresponding Author: Peerapol Sukon, Faculty of Veterinary Medicine, Khon Kaen University, 40002 Khon Kaen, Thailand

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Direct-fed microbial (DFM) products are defined as products containing live (viable) microorganisms (bacteria and/or yeast) (https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074707.htm) and have been under extensive investigation as potential replacements for antibiotics as growth promoters in livestock species, including broiler chickens[1]. DFM may be considered beneficial microorganisms[2] or probiotics, which confer a health benefit on the host when administered in adequate amounts (http://www.fao.org/3/a-a0512e.pdf). Although the exact mechanisms for these health benefits have not been fully understood, several beneficial mechanisms for DFM or probiotics have been proposed, including modification of the gut microbiota, modulation of the immune system, production of antimicrobial substances, competitive exclusion for pathogens on the gut mucosa and strengthening of the gut epithelial barrier[3,4]. The gut of the broiler chicken is a complex organ, composed primarily of the small intestine (duodenum, jejunum and ileum), which interacts with tremendous quantities of gut microbiota. The mucosa where nutrient absorption occurs consists of epithelial lining cells resting on specialized mucosal structures called "villus" and "crypt". The villus is a repetitive formation protruding into the intestinal lumen; the crypt, located on each side of the villus, is an invagination of the epithelium. In chicks, the development of the villus and crypt is clearly observed in the post-hatch period[5,6]. The villus is considered to be a specialized structure for increasing absorptive surface area. Therefore, in many nutritional trials in broiler chickens, the villus height (VH) is measured histologically to indicate an increased surface area for nutrient absorption[7,8]. The results from previous studies of DFM on intestinal villus height showed controversial evidence. Many studies showed that supplementation with DFM was associated with increased VH or increased villus height-to-crypt depth (VH/CD) ratio in broiler chickens[7-11] but some studies did not[12,13]. Therefore, this systematic review and meta-analysis was conducted to evaluate the overall effect of DFM on mucosal intestinal morphology in broiler chickens and to explore factors associated with result heterogeneity.

MATERIALS AND METHODS

Review protocol: To minimize bias during the course of this study, a study protocol was developed using SYRCLE's protocol format. The protocol of this study is available as supporting information. Deviation from the protocol was indicated in the relevant sections.

Information sources and search strategy: Relevant studies were identified from the PubMed, Scopus, Poultry Science Journal and Google Scholar databases with the keywords: probiotic, direct-fed microbial, intestine and chickens. An example of search algorithm from PubMed is as follows: ((("probiotics" [MeSH Terms] OR "probiotics" [All Fields] OR "probiotic" [All Fields]) OR (direct [All Fields] AND fed [All Fields] AND microbial [All Fields])) OR ("intestines" [MeSH Terms] OR "intestines" [All Fields] OR "intestine" [All Fields])) AND ("chickens" [MeSH Terms] OR "chickens" [All Fields] OR "chicken" [All Fields]). This study was limited to articles in the English language. The last search was completed on May 19, 2016.

Eligibility criteria and study selection: Study eligibility was assessed with two screening steps by two independent reviewers. In the first step, the title and abstract was assessed. If the title and abstract passed the first screening, the full-text was assessed. The criteria of study selection for included studies were as follows: (1) Randomized or non-randomized controlled trials using DFM as intervention in broiler chickens, (2) Outcome reporting containing VH, CD or VH/CD ratio in duodenum, jejunum or ileum of the small intestine. Studies were excluded with the following criteria: (1) Reviews and duplicated articles, (2) Not about broiler chickens, (3) Trials with no control groups, *in vitro* or *in ovo* studies, (4) Non-viable DFM or treatments other than DFM (e.g., prebiotic, symbiotic, enzymes) or a combination of DFM with other products, (5) Incomplete outcomes data report and (6) Trials with disease challenges or antimicrobial products. The PRISMA flow chart of systematic review was used to summarize the study selection.

Study characteristics and data extraction: Two independent reviewers extracted the available data from the included studies. In each study, the following information was collected: (1) Bibliographic data (author's name, publication year), (2) Study design (number of animals, number of treatments and replications, number of birds/pen or block), (3) Animal model characteristics (broiler breed, sex, number of samples for histological outcomes/group (n), (4) Intervention characteristics (DFM species and strains, product classifications, administrative doses, application

frequency and route, duration of treatment) and (5) Outcome measurements (VH, CD or VH/CD ratio of duodenum, jejunum or ileum of the small intestine). Any inconsistencies were resolved by discussion.

Risk of bias assessment for included studies: Two independent reviewers screened and judged the risk of bias for the included studies using SYRCLE's risk of bias tool[14]. These reviewers screened and judged the risk of bias based on these criteria. The reported details of each included study were evaluated under 10 domains for risks of bias, including: Selection bias (domain 1, 2 and 3), performance bias (domain 4 and 5), detection bias (domain 6 and 7), attrition bias (domain 8), reporting bias (domain 9) and other (domain 10)[14]. Each domain was sorted into three categories: "yes" was indicated for the low risk of bias, "no" for the high risk of bias and "?" for the unclear risk of bias.

Outcome measures and statistical analysis: The primary outcome was a standardized mean difference (SMD) of VH in the small intestine; the secondary outcome was an SMD of CD and of VH/CD ratio. SMD was calculated as a mean divided by its standard deviation (SD). Comprehensive Meta-Analysis software version 3 (Biostat, Englewood, NJ, USA) was used to analyze the data. If the studies reported standard error (SE) or standard error of mean (SEM) values for the outcomes, these values were converted to SD. A random effects model was used to analyze the outcomes to obtain an overall or individual effect size with 95% confidence intervals[15]. Heterogeneity of included studies was evaluated using the Q statistic and quantified via the I^2 statistic. The percentage of I^2<25%, 50-75% and >75% indicated low, medium and high heterogeneity, respectively[16]. Subgroup analyses were planned a priori for some characteristics (broiler breed, sex, DFM product, microbial species, duration of treatment and application route). To reduce the number of subgroups and make subgroup interpretation easy, the outcomes from each intestinal segment were combined to represent the overall small intestine of the broiler chickens. Subgroup analyses were performed only if each subgroup contained at least 4 studies[17]. Sensitivity analyses were conducted to assess the robustness of the results based on decision making (model selection (random versus fixed), study inclusion (effect of an individual study), outcome measurement (pooling outcomes across intestinal segments versus not pooling)) during the process of the systematic review. Publication bias was assessed visually via funnel plot and was tested formally using Egger and Begg's test, with a p-value <0.10 indicating publication bias[18]. Cumulative meta-analysis over time was also conducted, although it was not planed in the protocol.

RESULTS

Search results and study selection: A total of 15987 citations published between 1914-2016 were identified. Of 15987 citations, 5520 citations were duplicated and 10405 citations were excluded with justification after the titles and abstracts were screened (Fig. 1). As a result,

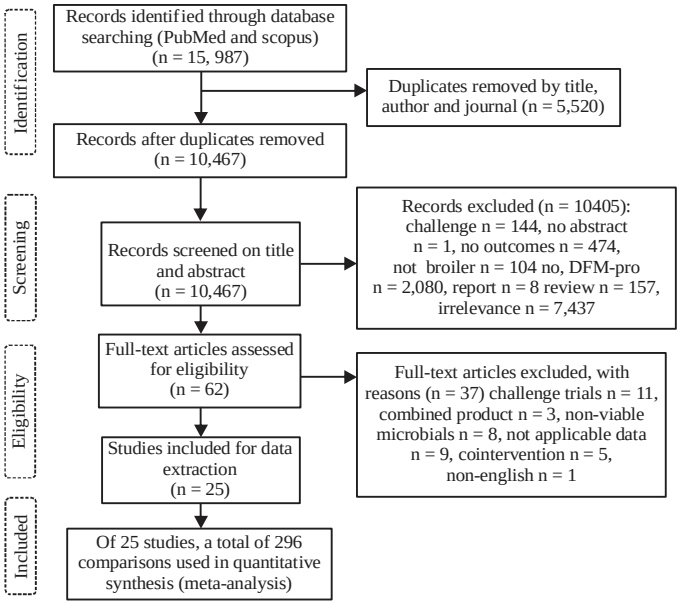

Fig. 1: A flow diagram of study selection

Table 1: Summary of effects of DFM supplementation on intestinal mucosal morphology

		Effect size[b]			Heterogeneity		
Outcomes	n[a]	SMD	95% CI	p-value	Q	p-value	I^2
VH							
Duodenum	37	3.59	2.33, 4.85	<0.001	409.85	<0.001	91
Jejunum	27	3.97	2.57, 5.36	<0.001	308.07	<0.001	92
Ileum	49	2.94	1.93, 3.94	<0.001	649.43	<0.001	93
Overall	113	3.38	2.69, 4.06	<0.001	1385.80	<0.001	92
CD							
Duodenum	32	0.05	-0.90, 0.99	0.924	302.25	<0.001	90
Jejunum	22	1.33	0.02, 2.63	0.046	245.54	<0.001	91
Ileum	42	-0.76	-1.56, 0.05	0.065	441.29	<0.001	91
Overall	96	-0.10	-0.66, 0.45	0.714	1018.88	<0.001	91
VH/CD ratio							
Duodenum	31	3.02	1.84, 4.20	<0.001	346.60	<0.001	91
Jejunum	21	2.51	1.50, 3.51	<0.001	172.33	<0.001	88
Ileum	35	2.73	1.60, 3.87	<0.001	446.71	<0.001	92
Overall	87	2.73	2.09, 3.36	<0.001	966.00	<0.001	91

CD: Crypt depth, CI: Confidence interval, SMD: Standardized mean difference, VH: Villus height, VH/CD: Villus height-to-crypt depth ratio, n[a]: Number of comparisons, [b]Effect sizes were estimated from a random effects model

62 full-text articles were assessed for eligibility. Of these, 25 articles (with a total of 296 comparisons) were included for data extraction and meta-analysis[7,10,12,13,19-39].

Study characteristics of included studies: The detailed characteristics of all included studies were described. The 25 included studies were published from 2005-2016 and had a total of 296 comparisons. Of these 25 studies, almost all studies (24 studies) reported randomized controlled trials. However, reports of blind outcome assessment were found in only one study. Ross was the most studied breed (16/25 studies). Male chicks were frequently used in the experiments (14 studies) and 11 studies did not report the sex of the experimental chicks. Commercial DFM products were used in 13 studies. The concentrations of microbials used ranged from 10^5-10^{10} CFU kg^{-1} and the microbials were typically administered mixed into feed (23 studies). A duration of treatment of $\leq$21 days was found in 4 studies, while 21 studies showed a treatment duration of >21 days. Of the 25 studies, 15 studies reported outcome measurements for duodenum, 12 studies for jejunum and 25 studies for ileum.

Primary analysis: The results of meta-analysis for the VH, CD and VH/CD ratio between the DFM and control groups were summarized in Table 1.

Effect of DFM on VH: Supplementation of DFM was associated with increased VH in the overall small intestine of broiler chickens (SMD = 3.38, 95% CI [2.69, 4.06], p<0.001, n = 113 comparisons) with high heterogeneity (Q = 1385.80, p<0.001, I^2 = 92%). Overall effects of DFM on VH in each small intestinal segment (duodenum, jejunum and ileum) were reported in Table 1. In the duodenum, of 37 comparisons, 23 comparisons favored DFM but only 5 comparisons favored the control (Fig. 2). In the jejunum, of 27 comparisons, 19 comparisons favored DFM but only 2 comparisons favored the control (Fig. 3). In the ileum, of 49 comparisons, 32 comparisons favored DFM but only 6 comparisons favored the control (Fig. 4). There was no significant difference in the effect magnitude of DFM between small intestinal segments (p = 0.464).

Effect of DFM on CD: Compared with control groups, DFM did not significantly alter CD in the overall small intestine of broiler chickens (SMD = -0.10, 95% CI [-0.66, 0.45], p = 0.714, n = 96 comparisons) with high heterogeneity (Q = 1018.88, p<0.001, I^2 = 91%). The overall effects of DFM on CD in each small intestinal segment (duodenum, jejunum and ileum) were reported in Table 1. A significant difference was seen in the impact of DFM on CD between small intestinal segments (p = 0.027). That is, although DFM did not significantly alter CD in the duodenum and ileum, DFM was associated with increased CD in the jejunum (SMD = 1.33, 95% CI [0.02, 2.63], p = 0.046, n = 22 comparisons). Forest plots of the magnitude of DFM's effect on the CD variable are available in the supporting information.

Effect of DFM on VH/CD ratio: Supplementation of DFM was associated with an increased VH/CD ratio in the

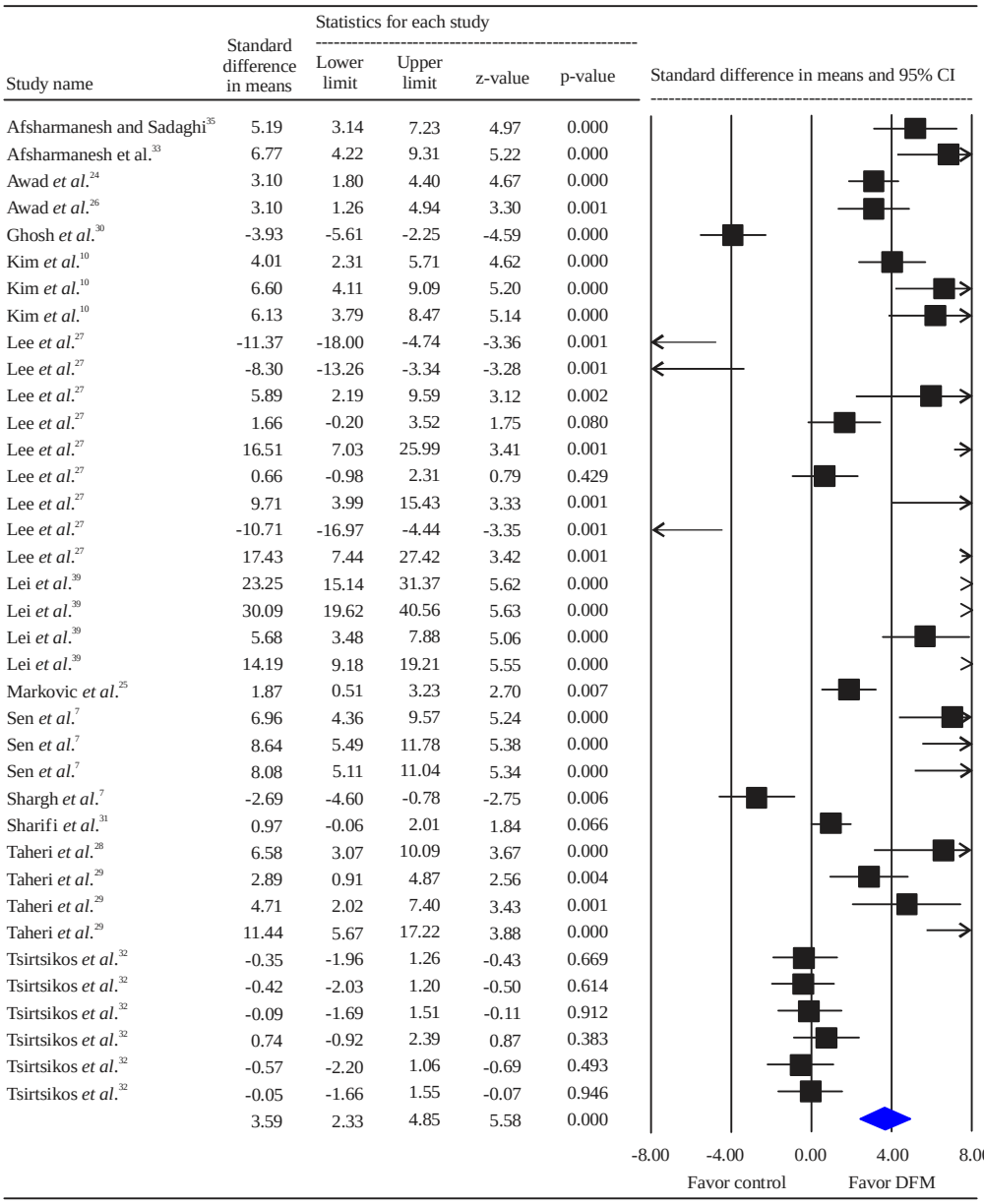

Fig. 2: Forest plot for the effect of DFM supplementation on villus height in duodenum of broiler chickens, calculations were based on a random effects model

overall small intestine of broiler chickens (SMD = 2.73, 95% CI [2.09, 3.36], p<0.001, n = 87 comparisons) with high heterogeneity (Q = 966.00, p<0.001, I^2 = 91%). The overall effects of DFM on the VH/CD ratio in each small intestinal segment (duodenum, jejunum and ileum) are reported in Table 1. There was no significant difference in the impact of DFM on the VH/CD ratio between small intestinal segments (p = 0.807). Forest plots of the magnitude of DFM's effect on the VH/CD ratio are available in the supporting information.

Subgroup analysis: Subgroup analyses for 6 characteristics (breed, sex, product, microbial species, duration of treatment and application route) were performed on the primary outcome, VH (Table 2). Significant differences among subgroups were seen in 5 characteristics: Breed, product, microbial species, duration of treatment and application route. For breed, the effect of DFM on the VH of the small intestine was greatest in Arbor Acres (SMD = 11.23, 95% CI [8.29, 14.17], p< 0.001, n = 14 comparisons). However, the effect was not statistically significant in Cobb (SMD = 0.42, 95% CI [-0.37,

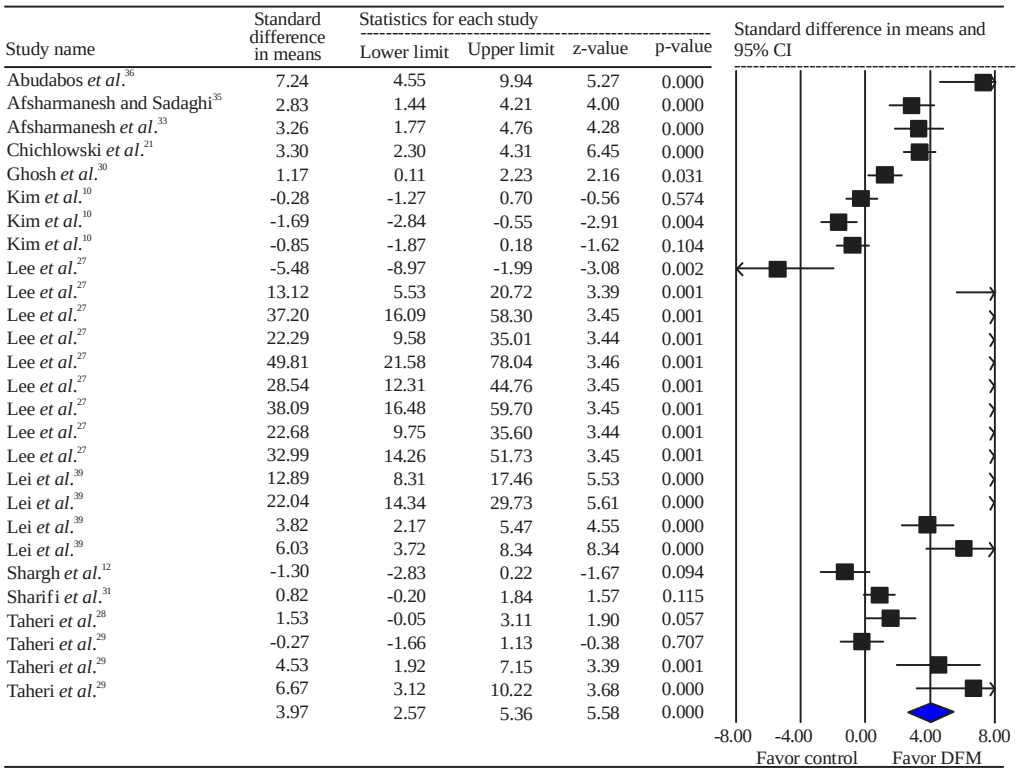

Fig. 3: Forest plot for the effect of DFM supplementation on villus height in jejunum of broiler chickens, calculations were based on a random effects model

Table 2: Subgroup analyses for the effects of DFM supplementation on VH of the small intestine in broiler chickens

		Effect size			Heterogeneity			p-value for subgroup
Subgroup	n[a]	SMD	95% CI	p-value	Q	p-value	I^2	difference
Breed[b]								
Arbor Acres	14	11.23	8.29, 14.17	<0.001	209.81	<0.001	94	<0.001
Cobb	14	0.42	-0.37, 1.21	0.293	45.20	<0.001	71	
Ross	53	3.09	2.25, 3.92	<0.001	672.53	<0.001	92	
Unknown	30	5.37	2.86, 7.88	<0.001	376.18	<0.001	92	
Sex								
Both sexes	4	3.28	1.44, 5.12	<0.001	13.10	0.004	77	0.921
Male	56	3.51	2.65, 4.38	<0.001	602.56	<0.001	91	
Unknown	53	3.23	2.10, 4.37	<0.001	742.53	<0.001	93	
Product								
Commercial	44	2.09	1.27, 2.92	<0.001	493.61	<0.001	91	<0.001
Non-commercial	42	4.71	3.61, 5.82	<0.001	610.41	<0.001	93	
Unknown	27	7.66	4.31, 11.01	<0.001	275.10	<0.001	91	
Species								
Multiple	44	1.58	0.88, 2.27	<0.001	383.07	<0.001	89	<0.001
Single	42	5.54	4.28, 6.80	<0.001	686.37	<0.001	94	
Unknown	27	7.66	4.31, 11.01	<0.001	275.10	<0.001	91	
Duration of treatment								
21≤	44	5.82	4.08, 7.56	<0.001	588.71	<0.001	93	0.002
>21	69	2.93	2.24, 3.61	<0.001	794.41	<0.001	91	
Application route								
Feed	81	3.42	2.72, 4.13	<0.001	1064.80	<0.001	92	0.005
Feed and gavage	27	7.66	4.31, 11.01	<0.001	275.10	<0.001	91	
Water	5	0.92	-1.39, 3.22	0.434	45.44	<0.001	91	

CI: Confidence interval, SMD: Standardized mean difference, n[a]: Number of comparisons, [b]Two comparisons of hybro breed were excluded from the subgroup analysis

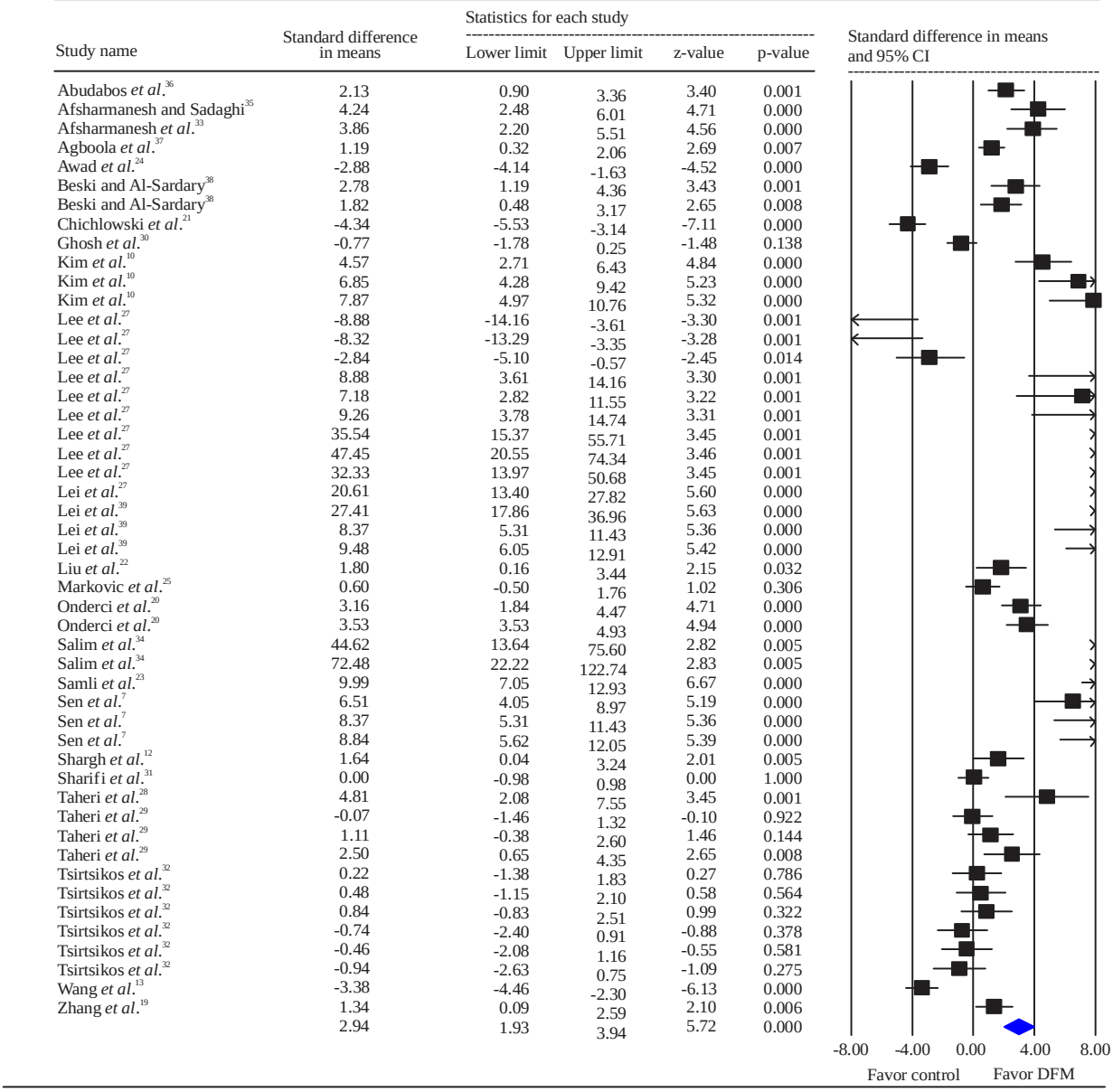

Fig. 4: Forest plot for the effect of DFM supplementation on villus height in ileum of broiler chickens, calculations were based on a random effects model

1.21], p = 0.293, n = 14 comparisons). For product, the commercial product was less effective (SMD = 2.47, 95% CI [1.29, 2.92], n = 44 comparisons) when compared with the non-commercial product (SMD = 4.71, 95% CI [3.61, 5.82], n = 42 comparisons) or with an unknown (unspecified) product (SMD = 7.66, 95% CI [4.31, 11.01], n = 27 comparisons). For microbial species, the effect of DFM on the VH of the small intestine was the greatest for unknown (unspecified) microbial species (SMD = 7.66 [95% CI [4.31, 11.01], n = 27 comparisons) when compared with single species (SMD = 5.54 [95% CI [4.28, 6.80], n = 42 comparisons) or multiple species (SMD = 1.58 [95% CI [0.88, 2.27], n = 44 comparisons). For duration of treatment and application route, although significant differences were observed between subgroups of these characteristics in a random effects model, such differences were not robust for a fixed effects model (p-value for subgroup difference = 0.102 for duration of treatment and = 0.791 for application route). A significant difference between subgroups was not seen for the sex of the birds.

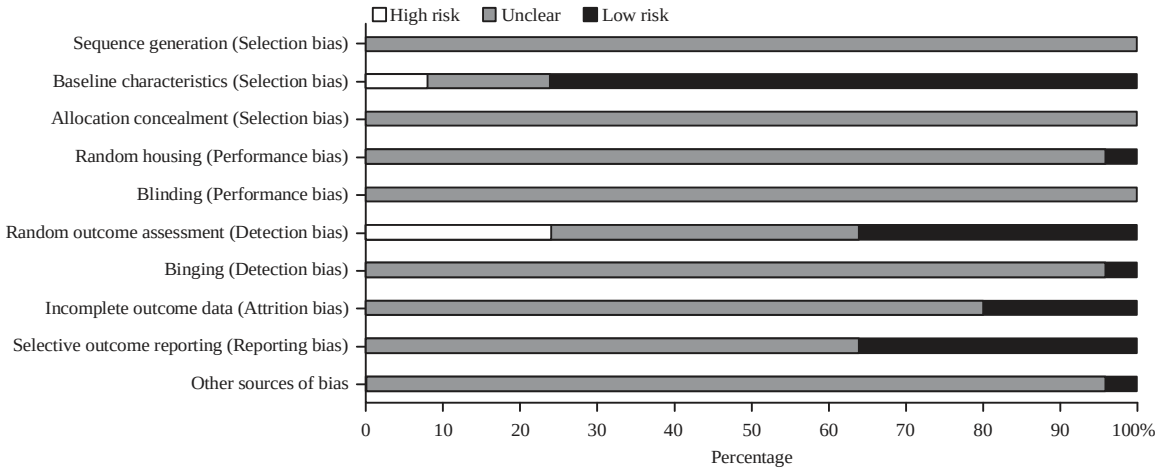

Fig. 5: Risk of bias graph presented as the percentage of 25 included studies

Sensitivity analysis: Detailed results from sensitivity analysis for VH (the primary outcome) are available in the supporting information. Compared with a fixed effects model, a random effects model resulted in a greater effect magnitude for all 3 segments of the small intestine, although the direction of the effect and the results from the statistical tests for effect magnitude did not change. The SMD for fixed effects versus random effects was 1.97 vs. 3.59 (n = 37 comparisons) for duodenum, 1.30 vs. 3.97 (n = 27 comparisons) for jejunum, 1.04 vs 2.94 (n = 49 comparisons) for ileum and 1.04 vs. 3.38 (n = 113 comparisons) overall. The effect magnitude for VH did not change significantly when one study was sequentially removed from the analysis. When the effect magnitude for VH was compared between pooled outcomes across intestinal segments and the outcomes for separated segments, the extent of the effect did not change significantly.

Risk of bias assessment: The overall results of the risk of bias assessment for each domain were presented in Fig. 5. All 25 articles reported unclear sequence generation (domain 1), allocation concealment (domain 3) and blinding (domain 5). For baseline characteristics (domain 2), 19 articles (76%) reported low risk of bias. For random housing (domain 4), 24 articles (96%) reported unclear risk of bias. For random outcome assessment (domain 6), 10 articles (40%) reported unclear risk of bias and 9 articles (36%) reported low risk of bias. For incomplete outcome data (domain 8), 20 articles (80%) reported unclear risk of bias. For selective outcome reporting (domain 9), 16 articles (64%) reported unclear risk of bias and other 9 articles (36%) reported low risk of bias.

Publication bias: For the VH outcome of the overall intestinal segments (113 comparisons), publication bias was clearly observed (Fig. 6). Egger's test for publication bias showed statistically significant results ($p < 0.001$). Duval and Tweedie's trim and fill methods indicated 21 missing comparisons and including these missing comparisons resulted in an adjusted SMD of 2.31 (95% CI, 1.61-3.02), compared with the original, unadjusted SMD = 3.38 (95% CI, 2.70-4.05).

For the CD outcome of the overall intestinal segments (96 comparisons), publication bias was not observed graphically. Egger's test for publication bias showed no significant results ($p = 0.720$).

For the VH/CD ratio of the overall intestinal segments (87 comparisons), publication bias was observed graphically. Egger's test for publication bias was statistically significant ($p < 0.001$).

DISCUSSION

The results of this systematic review and meta-analysis indicated that the supplementation of DFM was associated with increased VH of the small intestine in broiler chickens. This statement was supported by the meta-analytic result that the SMD of VH was significantly greater in the DFM supplementation group than in the control group. In addition, the result of a cumulative meta-analysis over time for DFM effects on increasing VH has been consistent since 2010. The results of this systematic review were consistent with those of original studies in other livestock species. Several studies showed that supplementation of DFM was associated with increasing villus height in the intestines of ducks[40,41] and

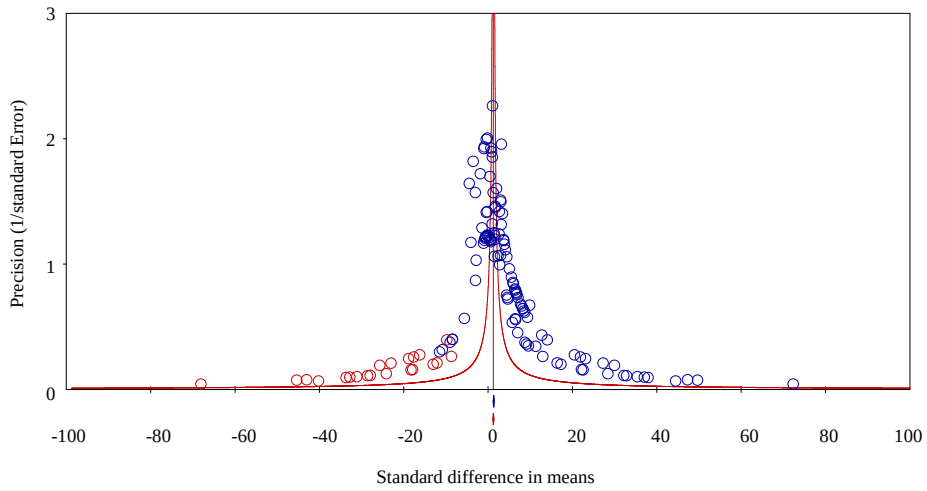

Fig. 6: Funnel plot for villus height from all intestinal segments, red circles indicated missing studies

pigs[42,43]. The length of VH is an indicator for the function of the intestinal villus[44]. The longer the VH is, the better the mucous secretion and nutrient absorption of the overall small intestine will be[45]. Although the exact mechanisms by which DFM or probiotics increase VH are still unknown, many mechanisms which may be indirectly associated with increasing VH have been proposed. For example, DFM may increase the number of normal micro-flora colonies found in the gastrointestinal tract of the animal host[46]. These micro-flora may then act to improve functionality through several pathways, including competing with harmful pathogens for energy and nutrients[47], promoting mucous secretion[46], preventing cytokine-induced epithelial damage[48], inhibiting pathogen adhesion on the intestinal epithelium, stimulating the immune system[49], producing bacteriocin and organic acids[45] and reducing pH levels in the intestinal tract and thus damaging harmful bacteria. These mechanisms together could suppress the colonization of the epithelium by pathogenic microorganisms and reduce the inflammation and infection of the intestinal mucosa[50], resulting in a longer villus and better gut health. It was assumed that better VH and VH/CD ratio could improve the nutrient digestibility and absorption capacity in the small intestine[51,52]. Conversely, shortening of a villus and a deeper crypt may lead to poor nutrient absorption and lower performance[53].

High heterogeneity was observed for all intestinal morphological outcomes. This result prompted subgroup analysis to explain possible sources of heterogeneity. From a priori subgroup analysis of 6 characteristics on VH outcome, significant differences among subgroups were seen in 5 characteristics: Breed, product, microbial species, duration of treatment and application route. However, a high degree of heterogeneity was still observed within all subgroups of 5 characteristics. These results indicated that other sources of bias may exist within each subgroup.

The robustness of the results of the meta-analysis was evaluated by sensitivity analysis. Although the direction of effect size for VH (the primary outcome) did not change between a random effects model (a priori model of choice in this study) and fixed effects model, the magnitude of the effect and its confidence interval were considerable greater in the random effects model than in the fixed effects model. A greater confidence interval for the random effects model could be explained by the random effects model's two sources of variance (within study variance and between study variance), while the fixed effects model has only one source of variance (within study variance)[54]. For the effect of an individual study, this study indicated that individual studies did not significantly affect meta-analytic results for VH because when sequential meta-analysis was performed with one study removed, the SMD of VH did not significantly change. In addition, this study indicated that the impact of VH was robust when pooling outcomes across intestinal segments compared with outcomes from separated segments.

In this systematic review and meta-analysis, the results of the risk of bias assessment from 25 included articles indicated lack of adequate reporting because a predominant unclear risk of bias was found. These results were consistent with those of other systematic reviews and meta-analyses from animal studies[55,56]. These can be explained by authors of animal trials who might not be aware of reporting bias domain or may not follow a specific guideline for reporting animal trials. Unlike animal trials, a guideline for reporting clinical trials in human

"called CONSORT statement or Consolidated Standards of Reporting Trials statement" was developed in 1996[57], first revised in 2001[58] and revised again in 2010[59]. This statement has helped to improve the reporting quality of human clinical trials over the past decade. As a result, at least two guidelines were developed in 2010 for use in reporting animal trials. These are the REFLECT statement (Reporting Guidelines for Randomized Controlled Trials in Livestock and Food Safety)[60] and ARRIVE guideline[61]. However, these guidelines are still not widely used, resulting in inadequate reporting. In addition, publication bias was clearly observed for the VH outcome. Taken together, these limitations indicated inferior or incomplete evidence for data synthesis.

CONCLUSION

It is concluded that the supplementation of DFM for broiler chickens was associated with increased intestinal villus height. However, this conclusion was based on the presence of heterogeneity, unclear risk of bias and publication bias of available data.

ACKNOWLEDGMENTS

The authors would like to thank the "Royal Scholarship under Her Royal Highness Princess Maha Chakri Sirindhorn Education Project" and the Faculty of Veterinary Medicine, Khon Kaen University, for financial support in this study.

REFERENCES

1. Hong, H.A., L.H. Duc and S.M. Cutting, 2005. The use of bacterial spore formers as probiotics. FEMS Microbiol. Rev., 29: 813-835.
2. Bernardeau, M. and J.P. Vernoux, 2013. Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion effects and health properties and consequences for extrapolation of farm animal results to humans. Clin. Microbiol. Infect., 19: 321-330.
3. Bermudez-Brito, M., J. Plaza-Diaz, S. Munoz-Quezada, C. Gomez-Llorente and A. Gil, 2012. Probiotic mechanisms of action. Ann. Nutr. Metab., 61: 160-174.
4. Buntyn, J.O., T.B. Schmidt, D.J. Nisbet and T.R. Callaway, 2016. The role of direct-fed microbials in conventional livestock production. Ann. Rev. Anim. Biosci., 4: 335-355.
5. Uni, Z., A. Geyra, H. Ben-Hur and D. Sklan, 2000. Small intestinal development in the young chick: Crypt formation and enterocyte proliferation and migration. Br. Poult. Sci., 41: 544-551.
6. Geyra, A., Z. Uni and D. Sklan, 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci., 80: 776-782.
7. Sen, S., S.L. Ingale, Y.W. Kim, J.S. Kim and K.H. Kim *et al*., 2012. Effect of supplementation of Bacillus subtilis LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. Res. Vet. Sci., 93: 264-268.
8. Ariyadi, B. and S. Harimurti, 2015. Effect of indigenous probiotics lactic acid bacteria on the small intestinal histology structure and the expression of mucins in the ileum of broiler chickens. Int. J. Poult. Sci., 14: 276-278.
9. Loh, T.C., N.T. Thanh, H.L. Foo, M. Hair-Beo and B.K. Azhar, 2010. Feeding of different levels of metabolite combinations produced by *Lactobacillus plantarum* on growth performance, fecal microflora, volatile fatty acids and villi height in broilers. Anim. Sci. J., 81: 205-214.
10. Kim, J.S., S.L. Ingale, Y.W. Kim, K.H. Kim and S. Sen *et al*., 2012. Effect of supplementation of multi microbe probiotic product on growth performance, apparent digestibility, cecal microbiota and small intestinal morphology of broilers. J. Anim. Physiol. Anim. Nutr., 96: 6118-626.
11. Giannenas, I., E. Tsalie, E. Triantafillou, S. Hessenberger, K. Teichmann, M. Mohnl and D. Tontis, 2014. Assessment of probiotics supplementation via feed or water on the growth performance, intestinal morphology and microflora of chickens after experimental infection with *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella*. Avian Pathol., 43: 209-216.
12. Shargh, M.S., B. Dastar, S. Zerehdaran, M. Khomeiri and A. Moradi, 2012. Effects of using plant extracts and a probiotic on performance, intestinal morphology and microflora population in broilers. J. Applied Poult. Res., 21: 201-208.
13. Wang, X., Y.Z. Farnell, E.D. Peebles, A.S. Kiess, K.G.S. Wamsley and W. Zhai, 2016. Effects of prebiotics, probiotics and their combination on growth performance, small intestine morphology and resident Lactobacillus of male broilers. Poult. Sci., 95: 1332-1340.
14. Hooijmans, C.R., M.M. Rovers, R.B. de Vries, M. Leenaars, M. Ritskes-Hoitinga and M.W. Langendam, 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol., Vol. 14. 10.1186/1471-2288-14-43
15. Hooijmans, C.R., J. IntHout, M. Ritskes-Hoitinga and M.M. Rovers, 2014. Meta-analyses of animal studies: An introduction of a valuable instrument to further improve healthcare. ILAR J., 55: 418-426.
16. Higgins, J.P., S.G. Thompson, J.J. Deeks and D.G. Altman, 2003. Measuring inconsistency in meta-analyses. Br. Med. J., 327: 557-560.

17. Hooijmans, C.R., R.B. de Vries, M.M. Rovers, H.G. Gooszen and M. Ritskes-Hoitinga, 2012. The effects of probiotic supplementation on experimental acute pancreatitis: A systematic review and meta-analysis. Plos One, Vol. 7. 10.1 371/journal.pone.0048811.
18. Egger, M., S.G. Davey, M. Schneider and C. Mider, 1997. Bias in meta-analysis detected by a simple, graphical test. Br. Med. J., 315: 629-634.
19. Zhang, A.W., B.D. Lee, S.K. Lee, K.W. Lee, G.H. An, K.B. Song and C.H. Lee, 2005. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality and ileal mucosa development of broiler chicks. J. Poult. Sci., 84: 1015-1021.
20. Onderci, M., N. Sahin, K. Sahin, G. Cikim, A. Aydin, I. Ozercan and S. Aydin, 2006. Efficacy of supplementation of alpha-amylase-producing bacterial culture on the performance, nutrient use and gut morphology of broiler chickens fed a corn-based diet. Poult. Sci., 85: 505-510.
21. Chichlowski, M., W.J. Croom, F.W. Edens, B.W. McBride and R. Qiu *et al*., 2007. Microarchitecture and spatial relationship between bacteria and ileal, cecal and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac and salinomycin. Poult. Sci., 86: 1121-1132.
22. Liu, J.R., S.F. Lai and B. Yu, 2007. Evaluation of an intestinal *Lactobacillus reuteri* strain expressing rumen fungal xylanase as a probiotic for broiler chickens fed on a wheat-based diet. Br. Poult. Sci., 48: 507-514.
23. Samli, H.E., N. Senkoylu, F. Koc, M. Kanter and A. Agma, 2007. Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Arch. Anim. Nutr., 61: 42-49.
24. Awad, W.A., K. Ghareeb, S. Abdel-Raheem and J. Bohm, 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. Poult. Sci., 88: 49-56.
25. Markovic, R., D. Sefera, M. Krstic and B. Petrujkic, 2009. Effect of different growth promoters on broiler performance and gut morphology. Arch. Med. Vet., 41: 163-169.
26. Awad, W.A., K. Ghareeb and J. Bohm, 2010. Effect of addition of a probiotic micro-organism to broiler diet on intestinal mucosal architecture and electrophysiological parameters. J. Anim. Physiol. Anim. Nutr., 94: 486-494.
27. Lee, K.W., S.H. Lee, H.S. Lillehoj, G.X. Li and S.I. Jang *et al*., 2010. Effects of direct-fed microbials on growth performance, gut morphometry and immune characteristics in broiler chickens. Poult. Sci., 89: 203-216.
28. Taheri, H.R., H. Moravej, A. Malakzadegan, F. Tabandeh, M. Zaghari, M. Shivazad and M. Adibmoradi, 2010. Efficacy of *Pediococcus acidlactici*-based probiotic on intestinal coliforms and villus height, serum cholesterol level and performance of broiler chickens. Afr. J. Biotechnol., 9: 7564-7567.
29. Taheri, H.R., H. Moravej, F. Tabandeh, M. Zaghari and M. Shivazad, 2010. Efficacy of combined or single use of *Lactobacillus crispatus* LT116 and *L. johnsonii* LT171 on broiler performance. Br. Poult. Sci., 51: 580-585.
30. Ghosh, T.K., S. Haldar, M.R. Bedford, N. Muthusami and I. Samanta, 2012. Assessment of yeast cell wall as replacements for antibiotic growth promoters in broiler diets: Effects on performance, intestinal histo-morphology and humoral immune responses. Anim. Physiol. Anim. Nutr., 96: 275-284.
31. Sharifi, S.D., A. Dibamehr, H. Lotfollahian and B. Baurhoo, 2012. Effects of flavomycin and probiotic supplementation to diets containing different sources of fat on growth performance, intestinal morphology, apparent metabolizable energy and fat digestibility in broiler chickens. Poult. Sci., 91: 918-927.
32. Tsirtsikos, P., K. Fegeros, C. Balaskas, A. Kominakis and K.C. Mountzouris, 2012. Dietary probiotic inclusion level modulates intestinal mucin composition and mucosal morphology in broilers. Poult. Sci., 91: 1860-1868.
33. Afsharmanesh, M., B. Sadaghi and F.G. Silversides, 2013. Influence of supplementation of prebiotic, probiotic and antibiotic to wet-fed wheat-based diets on growth, ileal nutrient digestibility, blood parameters and gastrointestinal characteristics of broiler chickens. Comparat. Clin. Pathol., 22: 245-251.
34. Salim, H.M., H.K. Kang, N. Akter, D.W. Kim and J.H. Kim *et al*., 2013. Supplementation of Direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population and ileal morphology of broiler chickens. Poult. Sci., 92: 2084-2090.
35. Afsharmanesh, M. and B. Sadaghi, 2014. Effects of dietary alternatives (probiotic, green tea powder and Kombucha tea) as antimicrobial growth promoters on growth, ileal nutrient digestibility, blood parameters and immune response of broiler chickens. Comparat. Clin. Pathol., 23: 717-724.
36. Abudabos, A.M., H.A. Al-Batshan and M.A. Murshed, 2015. Effects of prebiotics and probiotics on the performance and bacterial colonization of broiler chickens. S. Afr. J. Anim. Sci., 45: 419-428.
37. Agboola, A.F., B.R.O. Omidiwura, O. Odu, I.O. Popoola and E.A. Iyayi, 2015. Effects of organic acid and probiotic on performance and gut morphology in broiler chickens. S. Afr. J. Anim. Sci., 45: 494-501.
38. Beski, S.S.M. and S.Y.T. Al-Sardary, 2015. Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. Int. J. Poult. Sci., 14: 31-36.
39. Lei, X., X. Piao, Y. Ru, H. Zhang, A. Peron and H. Zhang, 2015. Effect of *Bacillus amyloliquefaciens*-based direct-fed microbial on performance, nutrient utilization, intestinal morphology and cecal microflora in broiler chickens. Asian-Aust. J. Anim. Sci., 28: 239-246.

40. Xing, Y., S. Wang, J. Fan, A.O. Oso and S.W. Kim *et al*., 2015. Effects of dietary supplementation with lysine-yielding on gut morphology, cecal microflora and intestinal immune response of Linwu ducks. J. Anim. Sci., 93: 3449-3457.
41. Kamollerd, C., P. Surachon, P. Maunglai, W. Siripornadulsil and P. Sukon, 2016. Assessment of probiotic potential of *Lactobacillus reuteri* MD5-2 isolated from ceca of Muscovy ducks. Korean J. Vet. Res., 56: 1-7.
42. Lee, S.H., S.L. Ingale, J.S. Kim, K.H. Kim and A. Lokhande *et al*., 2014. Effects of dietary supplementation with *Bacillus subtilis* LS 1-2 fermentation biomass on growth performance, nutrient digestibility, cecal microbiota and intestinal morphology of weanling pig. Anim. Feed Sci. Technol., 188: 102-110.
43. Dowarah, R., A.K. Verma, N. Agarwal, B.H.M. Patel and P. Singh, 2017. Effect of swine based probiotic on performance, diarrhoea scores, intestinal microbiota and gut health of grower-finisher crossbred pigs. Livest. Sci., 195: 74-79.
44. Shamoto, K. and K. Yamauchi, 2000. Recovery responses of chick intestinal villus morphology to different refeeding procedures. Poult. Sci., 79: 718-723.
45. Paul, S.K., G. Halder, M.K. Mondal and G. Samanta, 2007. Effect of organic acid salt on the performance and gut health of broiler chicken. J. Poult. Sci., 44: 389-395.
46. Cho, J.H., P.Y. Zhao and I.H. Kim, 2011. Probiotics as a dietary additive for pigs: A review. J. Anim. Vet. Adv., 10: 2127-2134.
47. Kamada, N., G.Y. Chen, N. Inohara and G. Nunez, 2013. Control of pathogens and pathobionts by the gut microbiota. Nature Immunol., 14: 685-690.
48. Sartor, R.B., 2006. Mechanisms of disease: Pathogenesis of Crohn's disease and ulcerative colitis. Nat. Clin. Pract. Gastroenterol. Hepatol., 3: 390-407.
49. Rolfe, R.D., 2000. The role of probiotic cultures in the control of gastrointestinal health. J. Nutr., 130: 396S-402S.
50. Deng, W., X.F. Dong, J.M. Tong and Q. Zhang, 2012. The probiotic *Bacillus licheniformis* ameliorates heat stress-induced impairment of egg production, gut morphology and intestinal mucosal immunity in laying hens. Poult. Sci., 91: 575-582.
51. Pluske, J.R., M.J. Tompson, C.S. Atwood, P.H. Bird, I.H. Williams and P.E. Hartmann, 1996. Maintenance of villus height and crypt depth and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. Br. J. Nutr., 76: 409-422.
52. Montagne, L., J.R. Pluske and D.J. Hampson, 2003. A review of interactions between dietary fibre and the intestinal mucosa and their consequences on digestive health in young non-ruminant animals. Anim. Feed. Sci. Technol., 108: 95-117.
53. Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan and M.Q. Wang, 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci., 82: 1030-1036.
54. Borenstein, M., L.V. Hedges, J.P.T. Higgins and H.R. Rothstein, 2009. Introduction to Meta-Analysis. John Wiley and Sons Ltd., West Sussex, England, pp: 421.
55. Li, J., P.Y. Hernanda, W.M. Bramer, M.P. Peppelenbosch, J. van Luijk and Q. Pan, 2015. Anti-tumor effects of metformin in animal models of hepatocellular carcinoma: A systematic review and meta-analysis. PLos One, Vol. 10. 10.1371/journal. pone.0127967.
56. Wever, K.E., M.H. Bruintjes, M.C. Warle and C.R. Hooijmans, 2016. Renal perfusion and function during pneumoperitoneum: A systematic review and meta-analysis of animal studies. studies. Plos One, Vol. 11. 10.1371/journal. pone.0163419.
57. Begg, C., M. Cho, S. Eastwood, R. Horton and D. Moher *et al*., 1996. Improving the quality of reporting of randomized controlled trials: The CONSORT statement. J. Am. Med. Assoc., 276: 637-639.
58. Moher, D., K.F. Schulz, D.G. Altman and L. Lepage, 2001. The CONSORT statement: Revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet, 357: 1191-1194.
59. Moher, D., S. Hopewell, K.F. Schulz, V. Montori and P.C. Gotzsche *et al*., 2010. CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. BMJ, Vol. 340. 10.1136/bmj.c869.
60. O'Connor, A.M., J.M. Sargeant, I.A. Gardner, J.S. Dickson and M.E. Torrence *et al*., 2010. The REFLECT statement: Methods and processes of creating reporting guidelines for randomized controlled trials for livestock and food safety. Prev. Vet. Med., 93: 11-18.
61. Kilkenny, C., W.J. Browne, I.C. Cuthill, M. Emerson and D.G. Altman, 2010. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biol., Vol. 8. 10.1371/journal.pbio.1000412.