Effect of Probiotic Containing Enterobacter Spp., Bacillus Spp., Cellulomonas Spp., Actinomyces Spp.Supplementation on The Laying Performance and Egg Cholesterol of Quail

by Widya Paramita

Submission date: 22-Nov-2019 12:04PM (UTC+0800)
Submission ID: 1219219834
File name: Bukti_C_45_Effect_of_Probiotic_Containing....pdf (622.31K)
Word count: 3187
Character count: 17596

IOP Conf. Series: Earth and Environmental Science **187** (2018) 012030 doi:10.1088/1755-1315/187/1/012030

Effect of Probiotic Containing *Enterobacter Spp., Bacillus Spp., Cellulomonas Spp., Actinomyces Spp.*Supplementation on The Laying Performance and Egg Cholesterol of Quail

W.P. Lokapirnasari[1*], A. R. Dewi[2], A. Fathinah[2], S. Hidanah[1] , N. Harijani[3], Soeharsono[4], K. Soepranianondo[1], T. Nurhajati[1], A.M.Sahidu[5],B. Karimah[2] and A.D. Andriani[2]

[1]Department of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Surabaya, Indonesia
[2]Veterinary Agribusiness, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Surabaya, Indonesia
[3]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Surabaya, Indonesia
[4]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Surabaya, Indonesia
[5]Department of Marine, Faculty of Fisheries and marine, Universitas Airlangga, Kampus C Mulyorejo, Surabaya, Indonesia

*Email: widyaparamitalokapirnasari@gmail.com

Abstract. The purpose of this study was to know the effect of probiotic supplementation to feed consumption, egg production, egg weight, feed conversion, feed efficiency and egg cholesterol. 80 quails of *Coturnixcoturnixjaponica* at 14 weeks of age were completely randomized into five treatments, each treatment consisted of four replication and each replication consisted by five heads. T0 as a control without probiotic supplementation, T1 with the addition of 1 ml probiotic/kg of feed, T2 with the addition of 2 ml probiotic/kg of feed, T3 with the addition 1 ml probiotic/liter drinking water and T4 with the addition of 2 ml probiotic/liter drinking water. The results showed that the probiotic supplementation gave a significant effect ($p<0,05$) to feed consumption, feed conversion, feed efficiency and egg cholesterol, but couldn't effect ($p>0,05$) egg weight and Quail Day Production. T4 showed the highest significantly different in feed conversion, feed efficiency and egg cholesterol. It could be better to give 2 ml probiotic containing *Enterobacteria spp., Bacillus spp., Cellulomonas spp.,* and*Actinomyces spp.* in drinking water for the best result.
***Keywords*:** Probiotic, production performance, egg cholesterol, quail

1. Introduction

Japanese quail, the smallest avian species is getting more importance for commercial egg and meat production with high rate of egg production. It has marked advantages such as fast growth, early sexual maturity, high rate of egg production and short generation interval [1].

Livestock feed r[11]adays become very expensive resulting in decrease in livestock production [2]. Recently the bane[26]n the use of antibiotic as a growth promoters [11]GP) in animal feeds, due to their residual effect in animal products as well as the development of bacter[10] resistance in animals and human body, have make a way to find alternatives of antibiotics [3]. It is generally accepted that direct-fed micr[28]als (DFMs), defined as a source of v[13]le microorganisms, as an alternative to antibiotics [4]. Probiotics or direct-fed microbials (DFM) are live microorganisms, when administered in adequate amounts, give a health benefit on the host [5]. Most probiotic strains which used in livestock are members of the *Bacillus* and *Enterococcus* genera [6].

Feeding Probiotic can reduce feed conversion [7]. In addition to increase the quantity of eggs produced, the fact that this probiotic can reduce egg cholesterol level may encourage people especially in developing countries to consume more eggs and enjoy good health as well as other benefits derived from egg [8].

The probiotic use in quail through feed and water, then expect that the consump[21]n of probiotics can improve the efficiency of feed, then increase egg production. The purpose of this study was to know the effect of probiotic containing *Enterobacteria, Bacillus spp., Cellulomonas spp. and Actinomyces spp.* supplementation to feed consumption, egg production, egg weight, feed conversion, feed efficiency and egg cholesterol.

2. Materials

The isolates of *Enterobacteria spp., Bacillus spp., Cellulomonas spp.,* and*Actinomyces spp.*isolated from bovine rumen fluid waste of Surabaya Abbatoir, Indonesia, from previous study [9]. Organic quail feed was made from organic grain and other crop without any antibiotic, chemical composition and other prohibited materials. A total of 80 laying quails (14 weeks) from Bojonegoro, Indonesia.

3. Methodology

3.1 Study Area and Farm Management

The research was conducted in the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. Probiotics used in this study contained cellulolytic and proteolytic bacteria consisted *of Enterobacteria, Bacillus spp., Cellulomonas s*[25] and*Actinomyces spp.*

A total of 80 laying quails (14 weeks) were randomly assigned to five treatments, with four replication each treatment and five heads each replication, the treatments were as follow: T0 was control with 100% quail feed, T1 was quail feed with 1 ml probiotic/gram of feed, T2 was quail feed with 2 ml probiotic/gram of feed, T3 was quail feed with 1 ml probiotic/liter water drinking and T4 was quail feed with 2 ml probiotic/liter water drinking

The probiotic liquid, dissolved in water (free chlorine and other antiseptics), then allowed to stand for 24 hours without aeration. The probiotic solution (T1 and T2) sprayed evenly into 100 kg of feed and then left the feed dried and the other probiotic solution (T3 and T4) then stirred evenly into drinking water.

3.2 D[18]a Collection and Statistical Analysis

Feed intake, egg production, egg weight were recorded daily during the experimental period that lasted [7]8 days. feed intake was a number of feed given divided with feed which not consumed. Egg weight was calculated by egg weight by egg production. Egg production was calculated in quail-day productio[14]QDP) with the ratio of eggs produced per day and the total of females quail in population [14]100%. Feed conversion was calculated as a ratio of feed intake by the egg weight. Feed efficiency was calculated as a ratio of egg weight by feed intake. The egg cholesterol measured after four week of treatment, with 5 randomized eggs each treatment, with enzymatic colorimetric method.

3.3 Statistical analysis

Data analysis was performed by Analysis of Variance (ANOVA). If the result showed differences or significantly different then continued with Duncan Multiple Range Test [10]. Statistical analysis performed with SPSS for Windows 21.0.

4. Results and Discussion

The results showed that the probiotic supplementation gave a significant effect ($p<0,05$) to feed consumption, feed conversion and feed efficiency where T4 was significantly the highest ($p<0.05$). Balanced gastrointestinal microflora were major functional effects attributed to the consumption of probiotics [11], then the utilization of selected probiotics might improve the metabolism of the host animals in various ways, there were absorptive capacity, protein metabolism and energy metabolism [12], then improved the digestion of food and minimized feed consumption[13]. This result in line with [14] reported that addition of probiotic to broiler chicken diets decreased feed conversion significantly. [15] reported that the supplementation of probiotics to laying hens improved hen performance, there were feed efficiency, egg production and egg quality. [16] reported that diets with probiotic supplemented showed a potential to improve feed efficiency. Moreover, the supplementation via drinking water might also contribute to the improvement, which consistent to previously studies that probiotic administration via drinking water appeared to be superior than in-feed supplementation method [17].The result showed in Table 1.

Tabel 1. The result of feed consumption, feed conversion, feed efficiency in 4 week of treatment

Treatment	Feed consumption (g) ± SD	Feed conversion ± SD	Feed efficiency ± SD
T0	23.0227 ^{e} ± 0.01673	2.1239 ^{c} ± 0.01321	47.5178 ^{a} ± 0.00837
T1	21.4060 ^{b} ± 0.02074	1.9644 ^{a} ± 0.01304	48.6541 ^{e} ± 0.01325
T2	22.0545 ^{c} ± 0.01581	2.0593 ^{b} ± 0.01140	50.5962 ^{c} ± 0.01893
T3	22.4333 ^{d} ± 0.01791	2.0579 ^{b} ± 0.01472	48.3588 ^{b} ± 0.01734
T4	21.3587 ^{a} ± 0.01304	1.9645 ^{a} ± 0.02550	50.3900 ^{d} ± 0.0342

$^{a, b c d e}$ different superscript in the same column showed a different effect ($p<0,05$)

The results showed that the probiotic supplementation couldn't effect ($p>0.05$) Quail egg weight and Quail Day Production, where the treatment showed lower egg weight and egg production. Egg production was costly in terms of energy and protein. The required energy for egg formation may be derived from daily feed intake. Daily feed intake was more important source of nutrient for small birds like quail than body reserved. If energy or protein was limited, birds could compensate by reduced egg size or the number of eggs laid [18]. The effectiveness of probiotic application might depend on factors such as microbial species composition (example single or multistrain), liveability, supplemental administration dose, method and frequency of application and diet composition [15]. Although egg weight was a highly heritable trait, the beneficial effect of probioti on egg weight may be attributable to a favorable environment in the intestinal tract, which might help to assimilate more nutrients [15].

This result contrast with [19] reported that egg production improved by approximately 50% on supplementation with 10^{7} cfu/g of probiotic under normal environmental condition. [20] reported that there were significantly improvements in egg production and egg quality when 500 mg of *B. subtilis* culture/kg was added to the diets. [21] observed significant ($P < 0.05$) improvements in feed efficiency and egg production when 20-week-old layer hens were fed a probiotic mixture that included *Lactobacillus* strains. Similarly, [22] reported that probiotic (BioPlus 2b) fed to 27-week-old Brown-Nick layers could increase egg production. [23] reported that *Lactobacillus salivarius*^{CB} + *Bacillus subtilis*, showed significantly increased egg production and daily egg yield as well as decreased feed conversion. [24] reported that the inclusion of 0·6% liquid metabolite combinations, produced from three *L. plantarum* strains, demonstrated the best effect in improving the hens' egg production. [25] reported that the inclusion of *Bacillus licheniformis* in the hen diet was

effective to increase egg production. [23] reported that supplementation of feed mixture with probiotics increased egg production. [23] reported that dietary supplementation of 0.01% probiotic improved egg production and egg quality.The result showed in Table 2.

Tabel 2. The result of egg weight and QDP in 4 week of treatment

Treatment	Egg weight (g) ± SD	Quail day production (%) ± SD
T0	10.9072^{d} ± 0.01140	55.7677^{e} ± 0.02074
T1	10.8276^{c} ± 0.15210	52.7273^{c} ± 0.01581
T2	10.7244^{a} ± 0.02074	41.8222^{a} ± 0.01140
T3	10.8485^{c} ± 0.02341	55.0300^{d} ± 0.01801
T4	10.7567^{b} ± 0.01346	50.0300^{b} ± 0.01721

The results showed that the probiotic supplementation gave a significant effect ($p<0,05$) to egg cholesterol. Probiotics were reported to have hypocholesterolemic [27]. Some lactic acid bacteria might secrete high-activity bile salt hydrolase (BSH) during metabolism, and this secretion could considerably affect cholesterol levels [28].

Probiotic supplementation might also played a good role to change the lipid metabolism as various studies showed that probiotics could reduce total cholesterol and triglyceride contents of egg yolk [29]. [30] reported that Probiotics reduced cholesterol concentrations in egg yolk. [22] reported that supplementation probiotic *Bacillus licheniformis* and *Bacillus subtilis* decreased egg yolk cholesterol. [19] reported that the cholesterol content of eggs produced by probiotic (*Lactobacillus* culture) fed hens was significantly lower by 15.3% and 10.4% when compared to those of the control hens at 24 and 28 weeks of age, respectively. Other researchers [16] observed also beneficial effects of the tested probiotic on egg quality such as a lower cholesterol content. The result showed in Table 3.

Tabel 3. The result of egg cholesterol in 4 week of treatment

Treatment	Egg cholesterol (mg/g) ± SD
T0	19.0222^{e} ± 0.01064
T1	6.7600^{a} ± 0.01876
T2	6.9302^{b} ± 0.02134
T3	13.5697^{d} ± 0.01924
T4	12.4480^{c} ± 0.01856

5. Conclusion

It can be conclude that the probiotic can't increase quail production and egg weight, but can decrease the feed consumption, feed conversion, egg cholesterol, then increase feed efficiency. The administration of 2 ml probiotic/liter drinking water show the best result.

Authors' Contributions

WPL designed the research. NH and SH helped in designing theresearch. ARD and AF carried out the data collection and assisted in manuscript preparation; WPL, BK and ADA collected materials for manuscript. All authors have read and approved the final manuscript.

Acknowledgements

The authors would like to thank the Dean of faculty of Veterinary Medicine, Universitas Airlangga that have funded this research.

References

[1] Ahmed Y E, Hamid I I, Ibrahim M T and Ali A S 2017 comparative growth and production between black and brown japanese quail (coturnix japonica) performance under sudan conditions *Journal of Science and Technology***18(1)**23-32

[2] Edache J A, Usman M U S A, Haruna E S, Esilonu J O, Jibrin I, Okpala E J and Dogo I G 2017Replacement value of guinea corn for maize in practical diet fed to quail (coturnix coturnix japonica) chicks *Animal Research International***2(2)**311-13

[3] Yang Y, Iji P and Choct M 2009 Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in feed antibiotics *World's Poultry Science Journal***65** 97-114

[4] Lee K W and Lillehoj H S 2017 An update on direct-fed microbials in broiler chickens in post-antibiotic era *Animal Production Science***57(8)** 1575-81

[5] Hill C, et al 2014 Expert consensus document. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic *Nat. Rev. Gastroenterol. Hepatol* **11** 506–14.

[6] Gaggia F, Mattarelli P and Biavati B 2010 Probiotics and prebiotics in animal feeding for safe food production *Int. J. Food Microbiol* **141** 15-28

[7] Gao P, Ma C, Sun Z, Wang L, Huang S, Su X, Xu J and Zhang H 2017 Feed-additive probiotics accelerate yet antibiotics delay intestinal microbiota maturation in broiler chicken. *Microbiome* **5(1)** 91

[8] Ezema C and Eze D C 2015 Probiotic effect of yeast (Saccharomyces cerevisiae) on hen-day egg performance, serum and egg cholesterol levels in laying chicken *Pakistan Journal of Nutrition***14(1)**44

[9] Lokapirnasari W P, Nazar DS, Nurhajati T, Supranianondo K and Yulianto A B 2015 Production and assay of cellulolytic enzyme activity of *Enterobacter cloacae* WPL 214 isolated from bovine rumen fluid waste of Surabaya Abbatoir, Indonesia *Veterinary World***8(3)** 367-71

[10] Kusriningrum R S 2008 *Research Planning* (Surabaya: Airlangga University Press Surabaya) p 15

[11] Guillermo T, Andrea L, Juan D L, Xochitl H V, Billy M H and Todd C 2015 Food-producing animals and their health in relation to human health *Microbial ecology in health and disease***26 (1)** 25876

[12] Salminen S and Isolauri E 2006 Intestinal colonization, microbiota, and probiotics *J. Pediatr.***149**115-20

[13] AstutiF K Busono W and Sjofjan O 2015Effect of liquid probiotics addition in feed to the production in broiler*J-PAL***6(2)** 99-104

[14] Talebi A, AmirzadehB, Mokhtari B and Gahri H 2008 Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens *Avian Pathology***37(5)** 509-12

[15] Panda A K, Rama Rao S S, Raju M V and Sharma S S 2008 Effect of probiotic (Lactobacillus sporogenes) feeding on egg production and quality, yolk cholesterol and humoral immune response of white leghorn layer breeders *Journal of the Science of Food and Agriculture***88(1)**43-7

[16] Mikulski D I, Jankowski J, NaczmanskiJ, Mikulska M and Demey V 2012 Effects of dietary probiotic (Pediococcus acidilactici) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens *Poultry science***91(10)** 2691-700

[17] [17] Torshizi MA K, Moghaddam AR, Rahimi S, Mojgani N 2010 Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response *Br. Poult. Sci.* **51** 178–84

[18] Brand H V D, Parameter H K and Kemp B 2004. Effects of housing system outdoor vs cages

and age of laying hens on egg characteristics *Br. Poult. Sci.***45** 745-52

[19] Ramasamy K, Abdullah N, Jalaludin S, Wong M and Ho Y W 2009 Effects of Lactobacillus cultures on performance of laying hens, and total cholesterol, lipid and fatty acid composition of egg yolk *Journal of the Science of Food and Agriculture***89(3)** 482-6

[20] Xu, C L, JiC, Ma Q, Hao K, Jin Z Y and Li K 2006 Effects of a dried Bacillus subtilis culture on egg quality *Poultry Science***85(2)** 364-8

[21] Kalavathy R, Abdullah N, Jalaludin S, Wong C M and Ho Y W 2005 Effects of Lactobacillus cultures on performance and egg quality during the early laying period of hens *Journal of Animal and Feed Sciences***14(3)** 537-47

[22] Kurtoglu F, Seker E, Coskun B, Balevi T and Polat E S 2004 Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol *Food additives and contaminants***21(9)** 817-23

[23] Zhang Z F and Kim I H 2013 Effects of probiotic supplementation in different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hens *Journal of animal science***91(10)**4781-7

[24] Choe D W, Loh T C, Foo H L, Hair-Bejo M and Awis Q S 2012 Egg production, faecal pH and microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced by Lactobacillus plantarum strains *British poultry science***53(1)**106-15

[25] Lei K., Li Y L, Yu D Y, Rajput I R and Li W F 2013 Influence of dietary inclusion of Bacillus licheniformis on laying performance, egg quality, antioxidant enzyme activities, and intestinal barrier function of laying hens *Poultry science***92(9)** 2389-95

[26] Arpášová H, Kačániová M, Pistová V, Gálik B, Fik M and Hleba L 2016 Effect of Probiotics and Humic Acid on Egg Production and Quality Parameters of Laying Hens Eggs *Scientific Papers: Animal Science & Biotechnologies/Lucrari Stiintifice: Zootehnie si Biotehnologii* **49(2)** 1-9

[27] Chiang S S and Pan T M 2012Beneficial effects of Lactobacillus paracasei subsp. paracasei NTU 101 and its fermented products *Applied Microbiology and Biotechnology***93(3)** 903–16

[28] Nguyen T, Kang J, Lee M 2007 Characterization of Lactobacillus plantarum PH04, a potential probiotic bacterium with cholesterol-lowering effects *International Journal of Food Microbiology***113(3)** 358-61

[29] Mohan B, Kadirvel R, Bhaskaran M, Natarajan M 1995 Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers*Br. Poult. Sci.***36**799

[30] Mahdavi A H, Rahmani H R and Pourreza J 2005. Effect of probiotic supplements on egg quality and laying hen's performance *Int. J. Poult. Sci.* **4(4)**488-92

Effect of Probiotic Containing Enterobacter Spp., Bacillus Spp., Cellulomonas Spp., Actinomyces Spp.Supplementation on The Laying Performance and Egg Cholesterol of Quail

ORIGINALITY REPORT

24%	15%	21%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Zhang, J. L., Q. M. Xie, J. Ji, W. H. Yang, Y. B. Wu, C. Li, J. Y. Ma, and Y. Z. Bi. "Different combinations of probiotics improve the production performance, egg quality, and immune response of layer hens", Poultry Science, 2012. Publication	3%
2	jwpr.science-line.com Internet Source	2%
3	www.aujst.com Internet Source	2%
4	SEZER, Metin. "Heritability of exterior egg quality traits in japanese quail", Nobel Tıp Kitapevi, 2007. Publication	2%
5	Xiaolu Liu, Hai Yan, Le Lv, Qianqian Xu, Chunhua Yin, Keyi Zhang, Pei Wang, Jiye Hu. "Growth Performance and Meat Quality of	1%

Broiler Chickens Supplemented with Bacillus licheniformis in Drinking Water", Asian-Australasian Journal of Animal Sciences, 2012
Publication

6	www.poultry.luminal-nutrition.com Internet Source	1%
7	www.tandfonline.com Internet Source	1%
8	Matias A. Michel, Fernando A. Revidatti, Ricardo J. Fernández, Martín L. Sindik et al. "Combination of a Lactobacillus-Based Probiotic and Organic Acids Decrease Egg to Chick Weight Loss and Reduce Salmonella spp. Counts in the Litter of Commercial Broiler Breeders", Food and Nutrition Sciences, 2019 Publication	1%
9	S Zhong, H Liu, H Zhang, T Han, H Jia, Y Xie. "Effects of Kluyveromyces marxianus Isolated from Tibetan Mushrooms on the Plasma Lipids, Egg Cholesterol Level, Egg Quality and Intestinal Health of Laying Hens", Revista Brasileira de Ciência Avícola, 2016 Publication	1%
10	www.publish.csiro.au Internet Source	1%

11	Darabighane Babak, Samuel N. Nahashon. "A Review on Effects of Aloe Vera as a Feed Additive in Broiler Chicken Diets", Annals of Animal Science, 2014 Publication	1%
12	Benjamin T Mayne, Shalem Y Leemaqz, Alicia K Smith, James Breen, Claire T Roberts, Tina Bianco-Miotto. "Accelerated placental aging in early onset preeclampsia pregnancies identified by DNA methylation", Epigenomics, 2017 Publication	1%
13	ujpsr.com Internet Source	1%
14	Sohail Hassan Khan, Muhammad Atif, Nasir Mukhtar, Abdul Rehman, Ghulam Fareed. "Effects of supplementation of multi-enzyme and multi-species probiotic on production performance, egg quality, cholesterol level and immune system in laying hens", Journal of Applied Animal Research, 2011 Publication	1%
15	Lihong Li, , Cheng Ji, and and Qiugang Ma. "INFLUENCE OF A DRIED BACILLUS SUBTILIS CULTURE ON THE PERFORMANCE AND EGG QUALITY IN LAYERS", Livestock Environment VII 18-20 May 2005 Beijing China, 2005.	1%

	Publication	
16	ps.fass.org Internet Source	1%
17	academic.oup.com Internet Source	1%
18	K-L. Chen. "Effect of roxarsone inclusion in the diet on the performance and hepatic lipid metabolism of laying Tsaiya duck", British Poultry Science, 7/2000 Publication	1%
19	link.springer.com Internet Source	<1%
20	www.pathogencombat.com Internet Source	<1%
21	documents.mx Internet Source	<1%
22	jbcr.co.in Internet Source	<1%
23	Payam Baghban-Kanani, Babak Hosseintabar-Ghasemabad, Saba Azimi-Youvalari, Alireza Seidavi et al. "Effects of Using *Artemisia annua* Leaves, Probiotic Blend, and Organic Acids on Performance, Egg Quality, Blood Biochemistry, and Antioxidant Status of Laying Hens", The Journal of Poultry Science, 2018 Publication	<1%

24	81.0.228.111 Internet Source	<1%
25	vri.cz Internet Source	<1%
26	scholar.sun.ac.za Internet Source	<1%
27	M Neijat, R B Shirley, J Barton, P Thiery, A Welsher, E Kiarie. "Effect of dietary supplementation of Bacillus subtilis DSM29784 on hen performance, egg quality indices, and apparent retention of dietary components in laying hens from 19 to 48 weeks of age", Poultry Science, 2019 Publication	<1%
28	Bomba, Alojz, Radomíra Nemcová, Ladislav Strojn_, and Dagmar Mudronˇová. "Probiotics for Farm Animals", Lactic Acid Bacteria Microbiological and Functional Aspects Fourth Edition, 2011. Publication	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		