Effect of bovine amniotic fluid administration on the intestinal bacterial composition and haematological parameters of broiler chickens

S Sugiharto, T Yudiarti, I Isroli, E Widiastuti and F D Putra

Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia
sgh_undip@yahoo.co.id

Abstract

Many efforts have been conducted to search for antibiotics substitute after the withdrawal of feed antibiotics. This study aimed to investigate the effect of bovine amniotic fluid (BAF) administration on the intestinal bacterial composition, haematological parameters and immune tissues development of broiler chickens. A hundred of 1-day-old male broiler chicks were allotted to control and the group receiving BAF (1 % in drinking water). At day 28, blood, immune organs and intestinal digesta were collected from the birds. Administration of 1% BAF in drinking water reduced ($P<0.05$) the number of coliform bacteria and increased ($P<0.05$) lactic acid bacteria (LAB) to coliform ratio in the intestine of birds. BAF administration increased the proportion of eosinophils ($P<0.05$) and heterophils ($P=0.09$) in the blood of broilers. The treatment also increased ($P<0.05$) the relative weight of Bursa Fabricius of broilers. In conclusion, BAF was potential to improve bacterial composition of the intestine and immune competence of broilers fed antibiotic-free diets.

Keywords: *bacterial composition, bovine amniotic fluid, broiler, blood profile*

Introduction

To control the infectious agents, the administration of sub-therapeutic antibiotics or antimicrobial agents has commonly been practiced in broiler diets. The growing concern of antimicrobial resistance in human has, however, led some countries to ban the use of sub-therapeutic antibiotics in animal feed. Given that the withdrawal of feed antibiotics may implicate in the performance and health problems (Sugiharto 2016), the search for alternative to feed antibiotics in broiler industries is of importance.

Amniotic fluid, which is the fluid surrounding the fetus, has been explored for decade, and it is now recognized that amniotic fluid contains multiple growth factors (Hirai et al 2002, Dasgupta et al 2016), antioxidants (Burlingame et al 2003), amino acids (glutamine and arginine) and minerals (zinc and iron) (Underwood and Sherman 2006). Amniotic fluid also contains immunoglobulins (Quan et al 1999) and lactoferrin (Underwood and Sherman 2006). The latter components (in amniotic fluid) may act as an antimicrobial agent against potential pathogenic microorganisms (Sugiharto et al 2015) in broiler chickens. To best of our knowledge, the use of amniotic fluid for poultry has never been reported. The present study therefore aimed to investigate the effect of amniotic fluid (bovine amniotic fluid, BAF) administration on the intestinal bacterial composition, haematological parameters and immune tissues development of broiler chickens fed antibiotics-free diets.

Methods

A total of 100 Lohman (MB-202) 1-day-old male broiler chicks (43.5 ± 0.25 g) were placed in an open-sided naturally ventilated broiler house, and randomly allotted to two experimental groups of 50 chicks each and 5 replicates of 10 chicks. The experimental groups included control group and the group receiving BAF (1 % in drinking water). The broiler house had concrete floor pens (with rice husk as bedding material) and was equipped with a tray feeder and manual drinker. The birds were fed diets in mash form (Table 1), which were formulated to meet or exceed the requirements recommended by the National Research Council (1994). Feed and water were provided ad libitum throughout the study period (28 days). The BAF was obtained from the cow at delivering the calf by collecting aseptically from the amniotic sac immediately after parturition. The BAF was then stored at –10°C until use.

Table 1. Ingredients and composition (as-dry basis) of treatment diet

Items	(%, unless otherwise noted)
Maize	54.0
Soybean meal	27.0
Fish meal	9.00
Rice bran	6.75
Broken rice	1.00
DL-methionine	0.23
L-lysine	0.06
Limestone	1.01
Dicalcium phosphate	0.20
Premix[1]	0.50
NaCl	0.25
Calculated composition:	
Metabolizable energy (kcal/kg)[2]	2,892
Crude protein	22.5
Crude fiber	3.52
Calcium	1.03
Phosphor	0.56
Methionine	0.66
Lysine	1.43

[1] *Mineral-vitamin premix provided the following (per kg of diet) Ca 2.250 g, P 0.625 g, Fe 3.570 mg, Cu 0.640 mg, Mn 5.285 mg, Zn 0.003 mg, Co 0.001 mg, Se 0.013 mg, I 0.016 mg, Vit A 375 IU, Vit D 150 IU, Vit E 0.080 mg.*

[2] *Values were obtained based on the formula according to Bolton (1967), in which metabolizable energy = 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] +2.5}*

For evaluation of haematological profile, blood was obtained from the birds' wing veins and collected in Ethylenediaminetetraacetic acid (EDTA)-containing vacutainers at day 28. The same birds as blood sampled were sacrificed after being weight and, immediately the immune organs were removed and weighed. Subsequently, the digesta was aseptically collected from the intestine for the microbiological analysis.

The counts of erythrocytes and leukocytes were determined with the dilution flask method, and a Bürker chamber was used to count corpuscles. A light microscope with an immersion lens was used to determine the differential leukocytes of broilers. Coverslip technique was applied when preparing blood smears. Heterophils to lymphocytes ratio (H/L ratio) was calculated by dividing the numbers of heterophils and lymphocytes.

Approximately 1 g of digesta was suspended in 10 mL peptone (Merck, Darmstadt, Germany) solution, homogenized and then serially diluted with the same medium before pour plating on the selective agar media. Presumptive coliform bacteria were determined on MacConkey agar (Merck) after aerobic incubation at 37°C for 24 hours. Lactic acid bacteria (LAB) were counted on de Man-RogosaSharpe (Merck) agar after anaerobic incubation at 37°C for 48 hours. LAB to coliform ratio was calculated by dividing the numbers of LAB and coliform bacteria.

Data were compared by means of the Student's t-test (SAS Inst. Inc., Cary, NC, USA). The experimental unit was the pen and results were considered statistically significant when *P<0.05*. Data are presented as means ± SD.

Results and Discussion

The bacterial composition of the intestine has been known to play an important role in the health of broiler chickens. Following the withdrawal of feed antibiotics, administration of BAF was expected to control the number of potential pathogenic bacteria and maintain the balanced intestinal microflora of broilers. Our present data showed that BAF administration was associated with the reduced (*P<0.05*) number of coliform bacteria and the increased (*P<0.05*) LAB to coliform ratio in the intestine of birds. However, BAF administration did not affect (*P>0.05*) the LAB population in the intestine of broilers (Table 2). The mechanism by which BAF decreased total coliform was largely unknown, but immunoglobulins and lactoferrin contained in BAF (Quan et al 1999, Underwood and Sherman 2006) seemed to be responsible. Taken together, the present finding suggested that BAF is a potential antimicrobial agent that may be administrated to broiler chickens fed antibiotic-free diets.

Table 2. Composition of bacteria in the intestinal digesta of broilers

	Control	BAF	*P value*
Total coliform (log cfu/g)	3.65 ± 0.79^{a}	2.41 ± 0.15^{b}	0.02
LAB (log cfu/g)	7.05 ± 0.50	7.10 ± 0.36	0.86
LAB to coliform ratio	1.99 ± 0.35^{a}	2.95 ± 0.27^{b}	<0.01

LAB: lactic acid bacteria
cfu: colony forming units
^{a,b} *Values with different letters within the same row were significantly different*

The haematological parameters have long been used as representative indicators of physiological conditions and health of chickens (Sugiharto et al 2016). In this study, BAF administration increased the proportion of eosinophils (*P<0.05*) and heterophils (*P=0.09*) in the blood of broilers (Table 3). The other blood parameters were not significantly affected by the administration of BAF in the drinking water. Eosinophils are type of white blood cells playing a role in the inflammation process and defence against parasites, while heterophils are the primary phagocytic leukocyte (Davis et al 2008). The increased numbers or proportions of these leukocyte types may therefore indicate the improvement of the immune competence of broilers, as also suggested by Chand et al (2014) when feeding zinc and ascorbic acid to broiler chicks. To date, no exact rationale behind the increased eosinophils and heterophils proportion in broiler treated with BAF. However, some amino acids in BAF such as glutamine and arginine probably promoted the proliferation of immune cells including eosinophils and heterophils (Li et al 2007). The positive effect on the immune cells was also most likely exerted by iron and zinc (Walker et al 2005) as well as antioxidant (Brambilla et al 2008) in the BAF.

Table 3. Haematological parameters of broilers

	Control	BAF	*P value*
Hemoglobin (g/dL)	7.36 ± 1.67	6.36 ± 1.10	0.10
Erythrocytes (10^{12}/L)	2.86 ± 0.58	2.38 ± 0.48	0.35
Hematocrit (%)	21.7 ± 4.44	20.3 ± 1.26	0.19
MCV (fl)	77.2 ± 17.4	81.3 ± 22.3	0.78
MCH (pg)	26.2 ± 6.65	27.5 ± 7.35	0.80
MCHC (g/dL)	33.8 ± 1.13	33.9 ± 0.45	0.89
Leukocytes (10^{9}/L)	19.2 ± 2.82	17.8 ± 8.17	0.76
Eosinophils (%)	0.80 ± 0.30^{a}	5.80 ± 3.49^{b}	0.02
Heterophils (%)	18.5 ± 7.85	26.0 ± 7.66	0.09

Lymphocytes (%)	61.4 ± 23.9	56.6 ± 12.3	0.59
Monocytes (%)	10.2 ± 5.75	7.20 ± 4.72	0.61
H/L ratio	0.32 ± 0.24	0.44 ± 0.17	0.10

MCV: mean corpuscular volume
MCH: mean corpuscular haemoglobin
MCHC: mean corpuscular hemoglobin concentration
H/L ratio: ratio of heterophils and lymphocytes
[a,b] Values with different letters within the same row were significantly different

It has widely been known that lymphoid organs play important roles in the defense of birds against pathogenic microorganisms (El-Katcha et al 2014). In this study, treatment with BAF increased ($P<0.05$) the relative weight of Bursa Fabricius of broilers (Table 4), which is a primary lymphoid organ in poultry. Our finding was concomitant with Chand et al (2014) who reported an increased weight of lymphoid organs when feeding zinc and ascorbic acid to broiler chicks. Overall, BAF administration may be expected to improve the immune responses and functionality of broiler fed antibiotic-free diets.

Table 4. Lymphoid organ of broilers

	Control	**BAF**	***P value***
Spleen (g/100 BW)	0.15 ± 0.06	0.17 ± 0.11	0.76
Thymus (g/100 BW)	0.33 ± 0.16	0.28 ± 0.10	0.60
Bursa Fabricius (g/100 BW)	0.16 ± 0.04[a]	0.21 ± 0.04[b]	0.04

BW: body weight
[a,b] Values with different letters within the same row were significantly different

Owing to the antimicrobial and immune-improving effects of BAF, it seems potential to use such fluid as an alternative to in-feed antibiotics for broilers. However, further performance and immuno-pathological studies on broiler chickens are needed to verify the growth-promoting and antibiotic-like effects of BAF. To date, no commercial BAF is available.

Conclusion

- Administration of 1% BAF in the drinking water was potential to improve bacterial composition of the intestine and immune competence of broilers fed antibiotic-free diets.

Acknowledgements

The authors sincerely thank Setyo, Anisa and Dita for their assistance during study.

References

Bolton W 1967 Poultry nutrition. MAFF Bulletin. 1967. No.174, (HMSO, London)

Brambilla D, Mancuso C, Scuderi M R, Bosco P, Cantarella G, Lempereur L, Di Benedetto G, Pezzino S and Bernardini R 2008 The role of antioxidant supplement in immune system, neoplastic, and neurodegenerative disorders: a point of view for an assessment of the risk/benefit profile. Nutrition Journal. 7:29.

Burlingame J M, Esfandiari N, Sharma R K, Mascha E and Falcone T 2003 Total antioxidant capacity and reactive oxygen species in amniotic fluid. Obstetrics & Gynecology. 101:756-761.

Chand N, Naz S, Khan A, Khan S and Khan R U 2014 Performance traits and immune response of broiler chicks treated with zinc and ascorbic acid supplementation during cyclic heat stress. International Journal of Biometeorology. 58:2153-2157.

Dasgupta S, Arya S, Choudhary S and Jain S K 2016 Amniotic fluid: Source of trophic factors for the developing intestine. World Journal of Gastrointestinal Pathophysiology. 7:38-47.

Davis A K, Maney D L and Maerz J C 2008 The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology. 22:760-772.

El-Katcha M I, El-Kholy M E, Soltan M A and EL-Gayar A H 2014 Effect of dietary omega-3 to omega-6 ratio on growth performance, immune response, carcass traits and meat fatty acids profile of broiler chickens. Poultry Science Journal. 2:71-74.

Hirai C, Ichiba H, Saito M, Shintaku H, Yamano T and Kusuda S 2002 Trophic effect of multiple growth factors in amniotic fluid or human milk on cultured human fetal small intestinal cells. Journal of Pediatric Gastroenterology and Nutrition. 34:524-528.

Li P, Yin Y L, Li D, Kim S W and Wu G 2007 Amino acids and immune function. British Journal of Nutrition. 98:237-242.

NRC 1994 Nutrient Requirement of Poultry, 9th edition, (National Academic of Sciences, Nutritional Research Council, Washington DC)

Quan C P, Forestier F and Bouvet J P. 1999 Immunoglobulins of the human amniotic fluid. American Journal of Reproductive Immunology. 42:219-225.

Sugiharto S 2016 Role of nutraceuticals in gut health and growth performance of poultry. Journal of the Saudi Society of Agricultural Sciences. 15:99-111.

Sugiharto S, Jensen B B, Jensen K H and Lauridsen C 2015. Prevention of enterotoxigenic *Escherichia coli* infections in pigs by dairy-based nutrition. CAB Reviews. 10:052.

Sugiharto S, Yudiarti T and Isroli I 2016 Haematological and biochemical parameters of broilers fed cassava pulp fermented with filamentous fungi isolated from the Indonesian fermented dried cassava. Livestock Research for Rural Development. 28:4.

Underwood M A and Sherman M P 2006 Nutritional characteristics of amniotic fluid. NeoReviews. 7:e310.

Walker C F, Kordas K, Stoltzfus R J and Black R E 2005 Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. American Journal of Clinical Nutrition. 82:5-12.

Received 11 September 2016; Accepted 17 January 2017; Published 1 May 2017

Go to top