Nutrient and bioactive potentials of clove and carrot as natural feed additive/water additive candidates for organic broiler chicken

J S Mandey, F R Wolayan, C J Pontoh and Y H S Kowel
Animal Husbandry Faculty, Sam Ratulangi University, Manado, North Sulawesi, Indonesia

Corresponding author: jetsm_fapet@yahoo.co.id

Abstract. Research was conducted to evaluate functional character of nutrients and bioactive of clove (Syzygium aromaticum) as feed additive and carrot (Daucus carota) as water additive on organic broiler chickens. Research was done by laboratory analysis to phytochemicals, proximate on chemical compounds, β-carotene, and antioxidant potency of clove and carrot. Data were analyzed by descriptive method. Phytochemical screening was positive in flavonoid, tannin, saponin and triterpenoid for clove, and positive in saponin and triterpenoid for carrot. Quantitative analysis found total flavonoid 0.17% (w/w) on carrot, phenol total 80.07% (w/w) on clove and 4.08% (w/w) on carrot. Beta-carotene on clove 1.43% and carrot 0.45%. Proximate analysis on clove were dry matter 82.59%, ash 7.23%, crude protein 7.53%, crude fat 4.48%, crude fiber 14.14%, Ca 2.287%, P 0.087%, gross energy 4652.04 Kcal; and on carrot were dry matter 88.27%, ash 11.84%, crude protein 9.21%, crude fat 4.74%, crude fiber 12.14%, Ca 1.362%, P 0.335%, gross energy 3414.66 Kcal. Carrot was high in protein and aspartic acid, and clove was high in amino acids serine and phenylalanine. It can be concluded that clove and carrot can be used as an alternative feed additive/water additive because of its nutrients and bioactive compounds.

1. Introduction
Consumer awareness about the health properties, safety and quality of food from animal products has increased the use of natural supplements in livestock production. Herbs, essential oils, spices, plant extracts and phytobiotics delivered a host of health benefits for animals. They improved animal performance and the quality animal products.

Feed additives derived from plants, also called phylogenics or phytobiotics or botanicals, can be included in animal diets to improve their productivity [1]. Among these natural additives, herbs, aromatic plants and their extracts have been examined due to their advantages over the antibiotics as growth promoters. They are residue free and generally recognized as safe [1, 2]. Herbs and spices can inhibit oxidative rancidity and delay the development of off-flavor in some products [3].

After the ban of antibiotic growth promoters as feed additives by the European Union due to cross-resistance against pathogens and residues in tissues, scientists have searched for alternatives to antibiotics. Aromatic plants and its phytochemicals are becoming more important due to their antimicrobial effects and the stimulating effect on animal digestive systems. In addition, they possess biological activities such as that of antioxidants [4] and as hypocholesterolemic [5], and stimulate
effect on animal digestive systems [6, 7], to increase production of digestive enzymes and improve utilization of digestive products through enhanced liver functions [8].

*Syzygium aromaticum* (synonym: *Eugenia cariophyllata*) commonly known as clove, is median size tree (8-12 m) from the Mirtaceae family native from the Maluku islands in east Indonesia. Clove has been used as an antiseptic [9]. In addition, it has appetizing and stimulating effect of digestion [9], and antioxidant [10]. Meanwhile, carrot (*Daucus carota*) is a root vegetable, usually orange, purple, red, white or yellow in color, with a crisp texture and a rich source of β-carotene and contains other vitamins, like thiamine, riboflavin, vitamin B-complex and minerals [11]. Up to 20% of the carrot and fruits juice wastes mixture could be included in broiler diets to effectively replace about 40% corn in the diet [12]. Access to different types of foraging material such as silages and carrots improved animal welfare [13]. The present study was attempted to assess the phytoconstituents to perform analyzing of various bioactive components of clove as feed additive, and carrot as water additive candidate for organic broiler chickens.

2. Material and methods

2.1. Material

The spice clove and carrot used in this research were obtained from the local market in Manado.

2.2. Methods

Phase I of the research was carried out by laboratory analysis, consisting of phytochemical, proximate, amino acids, beta-carotene, and antioxidant analysis of clove and carrot.

2.2.1. Phytochemical analysis. Phytochemical screening of clove and carrot for alkaloid was carried out by the Mayer, Wagner and Dragendorff methods, the saponin was carried out by the Forth method, the flavonoid was carried out by the Bate Smith-Metchalf method, and the anthraquinone was performed by the Brontrager test. Total phenol content was estimated as equivalent to gallic acid according to Singleton et al [14]. The total flavonoid content was estimated according to Sulaiman and Balachandran [15].

2.2.2. Proximate and amino acids analysis. Clove and carrot were analyzed to see the content of water, ash, crude protein, crude fat and crude fiber were carried out according to the standard method of AOAC [16] and amino acids analysis by HPLC method.

2.2.3. Beta-carotene analysis. Beta-carotene analysis was carried out with Thin Layer Chromatography Scanner (TLC). The principle was based on the process of moving or shifting substances at different speeds [17]. The value of the retention factor was calculated according to the formula as follows:

\[
R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}
\]

2.2.4. Antioxidant analysis. Antioxidant activity of was done analyzed using the DPPH (1,1-diphenyl-2-pikrilhidrazil) method [18] follows:

\[
\text{% Scavenging DPPH free radical} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100
\]

2.2.5. Statistic. Data were analyzed using descriptive analysis method.

3. Results and discussion

The results of the study can be seen in Table 1 and Table 2. Phytochemical screening by color visualization were positive in flavonoid, tannin, saponin and triterpenoid for clove, and positive in
saponin and triterpenoid for carrot. Quantitative analysis by spectrophotometry found total flavonoid 0.17% (w/w) on carrot, phenol total 80.07% (w/w) on clove and 4.08% (w/w) on carrot. Beta-carotene on clove 1.43% and carrot 0.45%. Carrot was high in protein (9.21%) and aspartic acid (92.79 ppm). And clove was high in amino acids serine (76.80 ppm) and phenylalanine (67.73 ppm).

Table 1. Phytochemicals and the potency of clove and carrot

| Phytochemicals: | Clove            | Carrot            | Unit            | Technique of Analyses |
|-----------------|------------------|-------------------|-----------------|-----------------------|
| Flavonoid       | positive         | negative          | -               | Spectrophotometry     |
| Alkaloid        | Wagner negative  | negative          | -               | Spectrophotometry     |
|                 | Mayer negative   | negative          | -               | Spectrophotometry     |
|                 | Dragendorf negative | negative          | -               | Spectrophotometry     |
| Tannin          | positive         | negative          | -               | Color Visualization   |
| Saponin         | positive         | positive          | -               | -                     |
| Quinone         | negative         | negative          | -               | -                     |
| Steroid         | negative         | negative          | -               | -                     |
| Triterpenoid    | positive         | positive          | -               | -                     |
| Total flavonoid | un               | 0.17%             | % (w/w)         | Spectrophotometry     |
| Total phenol    | 80.07            | 4.08%             | % (w/w)         | Spectrophotometry     |
| Tannin          | 0.42             | 0.04%             | %               | Titrimetric           |
| Quercetin       | 0.46             | un                | %               | HPLC                  |
| Toxicity LC50   | 168.27           | 16.10 ppm         | BSLT            |
| Beta-carotene   | 1.43             | 0.45%             | %               | TLC Scanner           |
| Antioxidant IC50-DPPH | <31.25    | 372.83 ppm        | Spectrophotometry |
| Antioxidant IC50-DPPH (Vitamin C standard) | 4.78          | 4.78 ppm          | Spectrophotometry   |

The tannin and saponin contents of clove in this research were lower than those reported by Sulaiman and Anas [15] about 10.12%, 23.86%, respectively. The low concentrations of anti-nutritional factors suggested that clove is a good source of food for human and animals. Even though this development is still relatively new, but can provide interesting opportunity.

The phenol total on clove in this research was high. Extensive research has proven that polyphenols, powerful antioxidants which are ubiquitous in plants, constitute a valuable supplement for animal diets. Those biologically active compounds improve the quality of animal products and contribute to animal health and performance [19]. For this reason, further research is required to investigate the efficacy of polyphenols in animal nutrition.

Polyphenols contain active ingredients. Most importantly, polyphenols are powerful antioxidants that prevent oxidative stress [20]. Despite those benefits, most polyphenols are not readily absorbed in the small intestine, and they accumulate in small quantities in bodily tissues [21]. Flavonoids are the largest group of polyphenols which can be further divided into six subclasses: anthocyanins, isoflavones, flavanones, flavonols, flavones and flavanols [22, 21]. The metabolism of polyphenols has not been fully explained. Only approximately 5–10% of polyphenols are absorbed and metabolized in biochemical pathways [23].

Aromatic plants, their extracts and essential oils contain a variety of functional bioactive compounds, which have possible applications in the food, feed, pharmaceutical and cosmetic industries [24]. Ertas et al. [25] reported that supplementation of 200 ppm essential oil mix (include oregano, clove and anise oils) in broiler diets significantly improved the daily live weight gain and feed conversion ratio during a growing period of 5 week.
Table 2. Proximate analysis and amino acids content of clove and carrot

| Parameter                   | Clove   | Carrot  |
|-----------------------------|---------|---------|
| Dry matter (%)              | 82.59   | 88.27   |
| Ash (%)                     | 7.23    | 11.84   |
| Crude protein (%)           | 7.53    | 9.21    |
| Amino acids (ppm):          |         |         |
| Aspartic acid               | n.a.    | 92.79   |
| Serine                      | 76.80   | n.a.    |
| Histidine                   | 22.43   | n.a.    |
| Glycine                     | 27.91   | n.a.    |
| Valine                      | 0.95    | n.a.    |
| Phenylalanine               | 67.73   | n.a.    |
| Isoleucine                  | 25.76   | n.a.    |
| Crude fat (%)               | 4.48    | 4.74    |
| Crude fiber (%)             | 14.14   | 12.14   |
| Ca (%)                      | 2.287   | 1.362   |
| P (%)                       | 0.087   | 0.335   |
| Gross energy (Calorie/g)    | 4652.04 | 3414.37 |

n.a. = not applicable

Supplementation of clove in the diet or drinking water of birds lead to impairment of their performance [26]. Azadegan et al. [27] suggested that the improvement of productive performance of broiler was due to present of active material in clove which stimulate the digestion and has antibiotic effect against organisms. This leads to a greater efficiency in utilization of feed and improve the growth performance.

Many studies have reported that clove was rich in trace mineral which essential for protein and carbohydrate metabolism. Reduced the synthesis of fatty acid and cholesterol, that could be improved broiler performance [8]. Adding of aqueous extract of clove flower at different concentrations led to improved productive and physiological effects of broiler chicken [28].

Total phenolic content of carrott in this research was 4.08% (w/w). Kulkarni [29] reported his research that ethanolic extracts from carrots showed total phenolic content of 58 mg/g dry plant material, and in screening phytochemical have positive in flavonoids, alkaloid, and terpenoid. The phytochemical analysis of carrot peel showed positive results for diterpenes, steroids, flavonoids, and phenolic acids in both the extracts. Saponins were present only in the methanolic extract of carrot peel [30].

Proximate analysis of carrot aerial parts was crude fiber 9.07%, crude protein 14.59%, crude lipid 10.37%. Percentage of antioxidant activity of different extracts in carrot aerial parts in ethyl acetate extracts EC50 86.29 of gallic acid (positive control) 211.14 [31]. The total phenol content calculated in carrots through this experiment is 95mg/gm of gallic acid equivalents [32].

Bioactive constituents of carrot may be beneficial to a vast number of consumers. It is rich in pro-healthy antioxidants both of lipophilic (carotenoids) and hydrophilic (phenolic compounds) characters [33]. Carrots are a good source of carbohydrates and minerals like Ca, P, Fe and Mg [34]. It has been used for treatment of anti-diarrhea, anti-infection, anti-high blood cholesterol, anti-inflammation, anti-fungal anti-bacteria and anti-cancer [35].

Carrot is rich in total flavonoids, total phenol and triterpenoids. Phytochemicals play a vital role against number of diseases; unlike pharmaceutical chemical these phytochemicals do not have any side effect. Biochemically carrot is a source of β-carotene, fiber, protein and many essential micronutrients and functional ingredients. The presence of high concentrations of biochemicals in carrot roots make them to inhibit free radical scavengers, and immuno-enhancers.
4. Conclusion
Phytochemical analysis of clove and carrot showed the presence of flavonoids, terpenoids, phenol, protein, amino acids, fiber, and functional ingredients. They have rich antioxidant property which can combat various diseases. It can be concluded that clove and carrot can be used as an alternative feed additive/water additive because of its nutrients and bioactive compounds. Further research is required to develop its mechanism of action in organic broiler chickens for healthy product.

Acknowledgement
The authors wish to thank to the Ministry of Research, Technology and Higher Education of Indonesia for their financial supported and encouragement.

References
[1] Windisch W, Rohrer E and Schedle K 2009 J Anim Sci 86 140–148
[2] Bresen A and Roura, E 2010 Anim. Feed Sci. Technol. 158 1–14
[3] Duke J 2002 Handbook of Medicinal Spices. CRC Press: Boca Raton, FL, USA.
[4] Placha I, Ryzner M, Cobanova K, Faixova Z and Faix S 2015 Polish J. of Vet. Sci. 18 741–749
[5] Craig W J 1999 Am. J. Clin. Nutr. 70 (Suppl) 491–499
[6] Akyildiz S and Denli M 2016 Scientific Papers. Series D. Animal Science. LIX 71–74
[7] Bozkurt M, Küçükyılmaz K, Çalı A U, Çınar M, Çabuk M and Alçicek A 2012 Arch. Geflügelk. 76 (2), S. 81–87
[8] Hernandez F, Madrid J, Garcia V, Oreno J and Megias M D 2004 Poult. Sci. 83 169–174
[9] Mukhtar M A 2011 J. of Basic and Appl. Sci. 5 49–51
[10] Gülçin I, Sat I G, Beydemir S, Elmas M and Küfreoglu O I 2004 J. Agri. Food Chem. 87 393–400
[11] Walde S G, Math R G, Chakkarvarthi A and Rao D G 1992 Indian Food Packer. 46 37–42
[12] Rizal Y, Mahata M E, Andriani M and Guoyao W 2010 Int. J. of Poult. Sci. 9 886–889
[13] Steenfeldt S, Kjaer J and Engberg R M 2007 British Poult. Sci. 48 454–468
[14] Singleton V L, Orthofer R and Lamuela–Ravento R M 1999 Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent In: Packer L (Eds). Methods in Enzymology (San Diego: CA Academic Press) pp 152–178
[15] Sulaiman C T and Balachandran I 2012 Indian J. of Pharm. Sci. 74 258–260
[16] AOAC 2000 Official methods of analysis (Washington DC: Association of Official Analytical
[17] Sudjadi 1983. Penentuan Struktur Senyawa Organik. (Bandung: Ghalia Indonesia)
[18] Moharram H A and Youssef M M 2014 Alex J. Fd. Sci. Tech. 11 31–42
[19] Lipiński K, Mazur M, Antoszkiewicz Z and Purwin C 2017 Ann. Anim. Sci. 17 41–58
[20] Paszkiewicz M, Budzynska A, Rozalska B and Sadowska B 2012 Post. Hig. Med. Dosw. 66 637–646
[21] Surai P F 2014 J. Anim. Physiol. An. 98 19–31
[22] D’Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C and Masella R 2007 Ann. Ist. Super. Sanita. 43 348–361
[23] Chiva–Blanch G and Visioli F 2012 J. Berry Res. 2 63–71
[24] Christaki, E., Bonos E, Giannenas I and Florou–Paneri P 2012 Agriculture. 2 228–243
[25] Ertas O N, Gülter T, Çiftçi M, Dalkılıç B and Gülçihan Simsek U 2005 Int. J. of Poult. Sci. 4 879–884
[26] Petrovic V, Marcincek S, Popelka P, Simkova J, Martinova M, Molnar L and Kovac G 2011 Anim. Physiol. and Anim. Nutr. J. 96 970–977
[27] Azadegan M, Hassanabadi A, Nassiri H and Kermanshahi H 2013 Iran. J. Appl. Anim. Sci. 4 117–122
[28] Al–Tabari A S, Zahira A, Al–Zuhairi, Abdulrazzaq M 2018 Bas. J. Vet. Res. 17 165–175
[29] Kulkarni C P 2017 Int. J. of Adv. Sci. and Res. 2 74–76
[30] Sheila John, Priyadarshini S, Monica S J and Arumugam P 2017 Int. J. of Food Sci. and Nutr. 2 23–26
[31] Ayeni E A, Abubakar A, Ibrahim G, Atinga V and Muhammad Z 2018. J. Herbmed. Pharmacol. 7 68–73
[32] Rachel Paul. Gayathri R, Vishnu Priya V 2017 Int. J. Pharm. Sci. Rev. Res. 45 34–36
[33] Leja M, Kaminska I, Kramer M, Maksylewicz–Kaul A, Kammerer D and Carle R 2013 Plant Foods Human Nutr. 68 163–170
[34] Sharma K D, Karki S, Thakur N S and Attri S J. of Food Sci. and Technol. 49 22–32
[35] Balasundram N. 2006 Food Chem. 99 191–203