Gut health maintenance in broilers: comparing potential of honey to antibiotic effects on performance and clostridial counts

¹*Jimoh, A. A., ¹Ayuba, U., ¹Ibitoye, E. B., ²Raji, A. A. and ³Dabai, Y. U.

¹Department of Theriogenology and Animal Production,

²Department of Veterinary Pathology

³Department of Veterinary Microbiology, Faculty of Veterinary Medicine,
Usmanu Danfodiyo University, Sokoto, Nigeria

Correspondence Tel:+2347035166498, E-mail: aajimoh1@gmail.com

Abstract

This study was conducted to investigate antimicrobial potentials of honey at controlling proliferation of Clostridium perfringens (Cp) and promoting growth in broiler chickens. Graded concentrations of honey solution (HS) were compared to 5.0% oxytetracycline (OTC). A total of 96 day-old White Hubbard strain broiler chicks were randomly distributed into four treatment groups of 24 chicks each in three replicates. The groups were designated as T1 for control (water only), T2 (1.5% HS), T3 (3.0% HS) and T4 (5.0 % OTC solution at 1.25g/liter). Data were collected weekly on feed intake, weight gain and efficiency of feed utilization (EFU) for six weeks. At 7th week, six birds per treatment were randomly selected, slaughtered and dissected, and 1g of caecal contents per bird was sampled into labeled sterile bottles. The samples were subjected to bacterial culturing, identification and colony counting. Results showed that feed intake was significantly ($p < 0.05$) depressed by 3.0% HS, but EFU improved at 1.5% HS. Although the additive had no significant effect on weight, highest weight gain (1.69 kg/b) was noticed at 1.5% HS. Colonies of Cp were reduced in honey and OTC-treated groups ($T2 = 5.2 \times 10^5$; $T3 = 5.0 \times 10^5$ and $T4 = 4.6 \times 10^5$ cfu g⁻¹) compared to the control ($T1$; 8.7×10^5 cfu g⁻¹). At 1.5% concentration, HS compared well with OTC as it reduced gut load of Cp and promoted growth in the broilers. In conclusion, addition of HS at 1.5% concentration in drinking water could therefore serve as an alternative to OTC to improve performance and reduce gut load of Cp in broilers

Keywords: Broiler; gut health; honey; clostridial count

Introduction

Clostridium perfringens is a toxin-producing bacterium found in the intestinal tract of healthy birds, farm animals and wildlife worldwide (Songer 1996; Craven *et al.*, 2003). Based on ability to produce the major lethal toxins α , β , E, I, five types (A-E) exist (Yoo *et al.*, 1997). The toxins are responsible for acute and subclinical necrotic enteritis (NE) in many species of poultry, especially in broiler and turkey flocks (Engstrom *et al.*, 2003). The subclinical NE is characterized by damage to intestinal mucosa, decrease in digestion, absorption and reduction in weight gain (Svobodova *et al.*, 2007). Increasing

incidence of NE in flocks raised without using antibiotics has been reported (Hafez, 2011). Antibiotics are used for prophylactic, curative and growth promotion purposes in the course of poultry production (Al-Bahry *et al.*, 2006). Most of antimicrobials are supplemented in poultry feeds at sub-therapeutic levels for reasons as, growth improvement, prevention or reduction of disease outbreaks, improving digestion, acceleration of weight gain and increase in feed conversion ratio (Donoghue, 2003). Aduku (2004) has classified antibiotics, hormones and antioxidants, as non-nutrient additives. Despite their benefits, Saikali and Singh

(2003) have raised concern regarding overuse of antibiotics in the course of producing food animals. Antibiotic residue in animals' products and its effects on human health are of major concern. Antibiotics have also been reported to disrupt the ecosystem of gastrointestinal flora and resulted in drug resistance (Karami *et al.*, 2006). Adoption of antibiotic-free production system where natural alternatives replace the use of in-feed antibiotics is a current area of research focus.

Honey is a unique, natural food, well known for its age long history of human consumption as source of nutrients and medicine (Bogdanov *et al.*, 2008; Ouchemoukh *et al.*, 2010). According to Dilnawaz *et al.* (1995), honey is a thick, translucent, pale yellow or yellowish brown liquid deposited in the honey comb by the bee, *Apis mellifera* Linn of the family Apidae. It has a characteristic pleasant odour and taste, and acidity (pH range) of 3.4-6.1. Honey exhibits antimicrobial, antiviral, antiparasitic, antimutagenic and anti-inflammatory properties ((Juszczak and Fortuna, 2006; Bogdanov *et al.*, 2008). And antioxidant activities of honey are reported in the prevention of chronic diseases (Ames *et al.*, 1993). Several studies have shown antibacterial activity of honey against *Escherichia coli*, *Campylobacter jejuni*, *Salmonella enterocolitis*, *Shigella dysenteriae* (Adebolu, 2005; Voidarov *et al.*, 2011), *Mycobacterium* (Asadi-pooya *et al.*, 2003), methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci (Cooper *et al.*, 2002; Al-waili *et al.*, 2005) and common gastrointestinal pathogenic bacteria (Lin *et al.*, 2011). The antibacterial potency of honey has been

attributed to its strong osmotic effect, naturally low pH (Kwakman and Zaat, 2012) and role of hydrogen peroxide (Kacaniova *et al.*, 2011). Honey has also been used for as growth promoter and for prophylactic purpose in animals (Juszczak and Fortuna 2006; Bogdanov *et al.*, 2008). It is on the basis of these potentials of honey that it is used as a substitute to OTC. This study was therefore aimed at comparing potential of honey to oxytetracycline in maintaining gut health against *Clostridium perfringens* and improving performance of broilers.

Materials and methods

Study area

This research work was carried out at the poultry pen of the Poultry unit of the Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto.

Sources and preparation of honey and oxytetracycline

The 5.0% OTC (powder) preparation used in the study was purchased from a sale-outlet in Sokoto metropolis. The recommended preventive dose of the OTC, 1.25g was weighed with Motler digital balance and dissolved in a litre of drinking water and served to the birds. As a measure against the use of adulterated product, the honey was sourced from a reputable farmer. Proximate analysis and phytochemical profile were assessed at the Soil science laboratory, Usmanu Danfodiyo University, Sokoto (Table 1). While preparing the honey solutions, 15mL and 30mL of the honey were measured and water was added to make 1litre solution, equivalent to 1.5 and 3.0% honey solution used in the study.

Table 1: Proximate composition and physicochemical profile of the Honey

Proximate	Values	Physicochemical properties	Values
Moisture	17.35 ± 2.05	pH	4.47 ± 1.93
Ash	0.34 ± 0.04	Soluble solid	80.96 ± 2.01
carbohydrate	81.16 ± 3.38	Total solid	82.65 ± 1.23
Protein	1.03 ± 0.25	Specific gravity	1.44 ± 0.10
Fat	0.12 ± 0.01		
Nitrogen Free Extract (NFE)			

Experimental setup and management of birds

A total of 96 day-old White Hubbard strain of broiler chicks sourced from Zaam Chicks Nigeria Limited, Kwara State, Nigeria, were used for the study. The birds were stabilized for a week, and then randomly allotted into four treatment groups containing 24 birds per treatment (i.e. eight birds in three replicates). Birds under treatment 1 (T1) received plain drinking water (control group). The birds under

treatments 2 (T2) and 3 (T3) received 1.5% and 3% honey solution respectively while those in treatment 4 (T4) were given containing 1.25g of OTC powder/ liter of water. Both the honey and the OTC were given at 3 days/week within the 3 weeks of the study. The birds were fed *ad-libitum* on formulated broiler starter for four weeks and broiler finisher from 5th week to the 7th week (Table 2). They were vaccinated against Newcastle disease and infectious bronchitis accordingly.

Table 2: Composition of the broiler starter and Finisher fed to the birds

Ingredients	Broiler starter	Broiler finiser
Maize	51.25	57.75
Soyabean	12.82	10.48
Groundnut cake	25.63	20.97
Wheat offal	6.00	7.50
Bone meal	1.50	1.00
Limestone	2.00	1.50
Salt	0.25	0.25
Lysine	0.20	0.20
Metionine	0.10	0.10
*Premix	0.25	0.25
Total	100.00	100.00
Calculated values		
Crude Protein	22.50	20.00
Metabolizable energy (kcal/kg)	2914.81	2976.02

*Slomix premix to supply Vitamin A (10,000mg), vitamin D (2,000mg), vitamin E (10mg), vitamin K₃ (2,000mg), Vit. B₁₂ (10,000mg), pantothenic acid (10,000mg), Niacin (26,000mg), folic acid (1,000mg), biotin (100,000mg), choline (150,000mg), Antioxidant (125,000mg), Manganese (10,000mg), Zinc (50,000mg), Cobalt (250mg), Iron (40,000mg), Copper (6,000mg), Iodine (500mg), Selenium (100mg).

Sampling and microbiology

The birds and feed were weighed weekly throughout the experimental period. Average weight gain and mean feed intake per treatment group in a week were calculated. Feed conversion ratio (FCR) for all the treatment groups were also calculated and recorded. At the end of the 7th

week, six birds from each treatment groups (two per replicate) were randomly selected and slaughtered by Halal method. The carcasses of the birds were dissected and about 2g each of ceecal content was collected with spatula and transferred into labeled sterile plastic bottles for each of the treatments. The samples were immediately

taken to the microbiology laboratory of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto for culture and identification of *Cp*.

One gram of each solid sample of the caecal contents was measure into a test-tube and diluted in a ratio of 1:9 (weight/volume) with sterile saline. One milliliter of each of the solutions was then subjected to 5 fold serial dilutions in a ratio of 1:9 (volume/volume) in sterile saline. And then 1mL from every fourth (10^4) dilution folds were inoculated into nutrient agar plates and then plated into blood agars base medium in which 5mL sheep blood had been added. The plates were transferred into anaerobic jar and incubated at 37°C for 24h using Gallenhamp incubator. The method used for the microbiological study was as described by Siragusa *et al.* (2008). On the following day, the plates were observed and areas of complete haemolysis caused by *Clostridium perfringens* colonies were also noticed on the blood agar media. The bacterial colonies were counted using electronic colony counter. The bacterium (*Clostridium perfringens*) was isolated and identified by the method described by Cheesebrough (2006), and the isolates were stained using Gram staining techniques. Cellular morphology of the colonies and their staining characteristics were used for

identification of the isolates. Biochemical tests were conducted including differential and catalase tests.

Data analysis and presentation

Data were subjected to one-way ANOVA at 5% probability using the parametric analytical tools of InStat Version 3.0 statistical software (Graph Pad, Software, Inc., San Diego, CA, USA). The data were presented in tabular form.

Results

The result on performance parameters is presented in the Table 3. There was a significant ($p < 0.05$) effect HS on the feed intake among the treatments. Feed intake decreased significantly in treatment 3, compared to treatments 1(control), 2 and 4. Highest feed intake (4.09kg/bird) was obtained in T4 but, the least feed intake (3.74kg/bird) was obtained in T3. Feed intake related inversely with concentration of honey. The result also revealed that highest body weight gain (1.69kg/bird) was recorded in birds under T2. while those given 3% honey solution (treatment 3) had the least body weight gain (1.39kg/bird) compared to the control group (1.58kg/bird). Efficiency of feed utilization (EFU) was not also significantly ($p > 0.05$) different among the treatment groups.

Table 3: performance indices of broilers treated with honey and oxytetracycline in drinking water

Parameters	T1	T2	T3	T4
Initial body weight (kg/b) at week 2	0.10	0.10	0.10	0.10
Final body weight (kg/b) at week 7	1.68	1.79	1.49	1.76
Weight gain (kg/b) within 6 weeks	1.58	1.69	1.39	1.66
Total feed intake (kg/b) within 6 weeks	4.03 ^b	3.94 ^b	3.74 ^a	4.09 ^b
Efficiency of Feed Utilization (EFU)	0.39	0.43	0.37	0.41

parameters with different superscripts are significantly different ($p > 0.05$)

KEY: T1= treatment 1 (control, only water), T2= treatment 2 (1.5% honey solution), T3= treatment 3 (3.0% honey solution), T4= treatment 4 (5.0% OTC at 1.25g/liter)

Antimicrobial action of the honey-additive was evaluated through inhibition of proliferation of *C. perfringens* in the ceaca as indicated by the bacterial colony counts. The result as presented in Figure 1, showed that 1.5% and 3.0% concentrations of honey solution significantly ($p < 0.05$) reduced *C. perfringens* load in the broilers from 8.70×10^5

10^5 cfu/ml ceaca content (T1, control group) to 5.20×10^5 and 5.00×10^5 cfu/ml ceaca content (i.e. 40.2% and 42.5% reduction in T2 and T3) respectively. However, OTC was best as it reduced the clostridal load to as low as 4.60×10^5 cfu/ml, equivalent to 47.1% reduction compared to that of control group.

Figure 1: *C. perfringens* counts ($10^5 \times \text{cfu/g}$) of intestinal content of broilers treated with honey and OTC in drinking water

Following staining, the isolates were gram-positive and subsequent microscopic examination revealed short, fat rods existing in cluster or in chain (Table 4). The

isolate was confirmed to be a member of gram negative enteric bacteria on triple sugar iron and catalase positive.

Table 4: Microbiological results for confirmation of the *Clostridium perfringens* isolates

Procedures/tests	Gram staining	Catalase test	Microscopy	TSI test
<i>C. perfringens</i>	+	+	Short rods in cluster	Confirmed

Discussion

There was a depressive effect on feed intake in the treatment group 3, i.e. birds that received 3% honey solution. The reduction in feed intake in the group was probably due to high energy content in honey (i.e. 82% carbohydrates, according to the USDA nutritional database). According to Jeffrey and Echazarreta (1996), simple sugars in honey are absorbed directly into the blood stream without undergoing any form of metabolism. This in effect might have

resulted into high blood sugar level (Hyperglycaemia), which may be signal to regulation of feed intake by the birds. Denbow (1994), has reported that feed intake in birds, as in mammals, is regulated both centrally and peripherally. Shurlock and Forbes (1981) have observed reduction in feed intake following infusion of glucose into the hepatic portal vein of fasted chickens. There was improvement in weight gain in birds under treatment 2 (1.5% honey solution) but depression in

weight of those treated with 3% honey solution. Although the observation was statistically not significant, it could be analyzed in the light of the established reports. Ahmed *et al.* (2012) had observed improved weight gain in broiler chicks when 10% honey was added in drinking water. In line with the finding of our study, the report of Ahmed *et al.* (2012) may indicate that younger birds respond better in term of weight gain to honey solution than adults. It may therefore be suggested that, using honey as additive may positively impact on body weight, when used at lower concentration. The depression in weight of the birds in T3 (Table 3), might not be unconnected to the gross reduction in their feed intake. This may advocate for further investigations of honey at lower concentrations. Efficiency of feed utilization, a ratio of live body weight gain to feed intake, is an index of how best the feed is utilized to carcasses. The higher the EFU value the better the feed utilization. In this study, the best EFU was recorded in the group (T2) treated with 1.5% honey solution. Extract of medicinal plants have been used as feed additives to improve weight gain and feed conversion ratio (Bozkurt *et al.*, 2009).

The improvement in feed utilization and the resultant body weight gain obtained in this study may also be related to antimicrobial effect of the honey-additive on the intestinal microbes. Antimicrobial effects of honey against common gastrointestinal pathogenic bacteria have been reported (Adebolu, 2005; Lin *et al.*, 2011). Ayanwale *et al.* (2006) reported that controlling gut microflora could positively influence poultry performance. In this study the treatment group with OTC solution had the lowest count (4.6×10^5 cfu), followed by 3.0% honey solution (5.0×10^5 cfu) and then 1.5% honey solution (5.2×10^5 cfu). The

control, however, had the highest counts of 8.7×10^5 cfu. In this regard, antimicrobial activity of honey may be described as bacteriostatic. Honey has been reported for its richness in hydrogen peroxide (Kacaniova *et al.*, 2011), strong osmotic effect and relatively low pH, which are reported to prevent growth of many bacteria (Kwakman and Zaat, 2012). Taormina *et al.* (2001), has reported that the non-peroxide constituent of honey is more important than its peroxide (H_2O_2) in terms of antibacterial effects.

Conclusion

The results of this study showed that honey improved growth performance of broilers in a concentration-dependent manner, optimally at 1.5% concentration, but reduced feed intake and weight of the birds at 3.0% concentration. The additive also competed satisfactorily with OTC at reducing caecal load of *C. perfringens* in broilers to very close counts. Meanwhile, more studies could be conducted to investigate lower concentrations of honey.

References

- Adebolu, T. 2005. Effect of natural honey on local isolates of diarrhea-causing bacteria in southwestern Nigeria. *African Journal of Biotechnology*, 4 (10):1172-1174.
- Aduku, A. O. 2004. *Animal nutrition in the Tropics: Feed and feeding, pasture management, monogastric and ruminant nutrition*, 1st edn, Pp 6, Davcon computers, Zaria, Nigeria. ISBN 9781257423
- Ahmed, M., Djebli, N., Hammaudi, S., Akila, B., and Hemida, H. 2012 Additive potential of ginger starch on antifungal potency of honey against *Candida albicans*, *Asian Pacific Journal of Tropical*

- Biomedicine*, **2** (4):253-255.
- Ayanwale, B. A., Kpe, M. and Ayanwale, V. A. 2006.** The effects of supplementing *Saccharomyces cerevisiae* in the diet on egg laying and egg quality characteristics of pullets. *International Journal of Poultry Science*, **5**(8):759-763.
- Al-Bahry S. N, AL-Mashani B. M, Elshafie A. E, Pathare N. and AL-Harthy A. H. 2006.** Plasmid profile of antibiotic resistant *E. coli* isolated from chickens intestines, *Alabama Journal of Academy sciences*, **77**:152-159.
- Al-waili N, Akmal M, AL-waili F, Salomn K. and Ali A. 2005.** The antimicrobial potential of honey from United Arab Emirates on some microbial isolates. *Medical Science Monitor*, **11**(12): 433-438.
- Ames, B. N., Shigenaga, M. K. and Hagen, T. M. 1993.** Oxidants, antioxidants, and the degenerative disease of aging. Proceedings of the National Academy of Sciences of USA, **90**: 7915-7922.
- Asadi-Pooya, A., Pnjehshahin, M., and Beheshit, S. 2003.** The antimycobacterial effect of honey: an *in vitro* study. *Rivstabiologia*, **66** (3): 491-496.
- Bogdanov, S., Jurendic, T., Sieber, R., and Gallmorin, P. 2008.** Honey for nutrition and health: A review. *Journal of the American College of Nutrition*, **27**(6): 677-689.
- Bozkurt, M., Kucukyilmaz, K. Catli, A. U. and Cinar, M. 2009.** Effect of dietary mannan oligosaccharide with or without oregano essential oil and hop extract supplementation on the performance and slaughter characteristics of male broilers. *South African Journal of Animal Science*, **39**: 223-232.
- Cheesbrough, M. 2006.** *District laboratory practice in tropical countries*. Cambridge University Press. P434.
- Cooper, R. Molan, P. and Harding, K. 2002.** The sensitivity to honey of gram positive cocci of clinical significance isolated from wounds. *In Journal of Applied Microbiology*, **13** (5):857-863
- Craven, S. E, Cox, N. A, Bailey J. S, Cosby, D. E. 2003.** Incidence and tracking of *Clostridium perfringes* through an integrated broiler chicken operation. *Avian Dis.* **47**: 77-711.
- Denbow, D. M. 1994.** Peripheral regulation of food intake in poultry. *Journal of Nutrition*, **124**:1349-1354.
- Dilnawaz, S, Shams-uz-zaman, S, Baqir, N. M, Rafi, S, and Ghulam, A. 1995.** Studies on the antimicrobial activity of honey, *Pakistan Journal of Pharmacological Sciences*, **8**(1): 51-62.
- Donoghue, D. J. 2003.** Antibiotic residues in poultry tissues and eggs: Human health concerns? *Journal of Poultry Science*, **82**(4): 618-621
- Engström B. E, Fermér C, Lindberg A, Saarinen E, Båverud V, Gunnarsson A 2003:** Molecular typing of isolates of *Clostridium perfringes* from healthy and diseased poultry. *Vet Microbiol.* **94**: 225-235.
- Hafez, H. M. 2011.** Enteric diseases of poultry with special attention to *Clostridium perfringens*, *Pakistan Veterinary Journal*, **31**(3): 175-184.
- Jeffrey, A. E. and Echazarreta, C. M. 1996.** Medicinal uses of honey. *Rev*

- Biomed.* 7: 43-49.
- Juszczak, L and Fortuna, T. 2006.** Rheology of selected polish honeys. *Journal of Food Engineering*, 75: 43-49
- Kacaniova, M., Vukovic, N., Bobkova, A., Fikselova, M., Rovna, K., Hascik, P., Cubon, J, Hleba, L, and Bobko, M. 2011.** Antimicrobial and antiradical activity of Slovakian honeydew honey samples. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(3): 354-36.
- Karami, M., Nowrouzian., F, Adelerberth, I., and Wold, A. E. 2006.** Tetracycline resistance in *E. coli* and persistence in the infertile colonic microbiota. *Antimicrobial agents and chemotherapy*, 50:156-161.
- Kwakman, P. and Zaat, S. 2012** Antibacterial component of honey. *IUBMB Life*, 64(1): 48-55.
- Lin S, Molan P., and Cursons, R. 2011.** The controlled *in vitro* susceptibility of gastro intestinal pathogen to the antibacterial effect of manuka honey. *European Journal of Clinical Microbiology and Infectious Diseases*, 30(4): 569-574.
- Ouchemoukh, S., Schweitzer, P., Bey, M. B, Djoudad-kadij H, and Louaileche H. 2010.** HPLC sugar profiles of Algerian honeys, *Food chemistry*, 121: 561-568.
- Saikali, Z and Singh, G. 2003.** Doxycycline and other tetracyclines in the treatment of bone metastasis. *Anti-cancer drugs*, 14: 773-778.
- Shurlock, T. G. H. and Forbes, J. M. 1981.** Evidence for hepatic glucostatic regulation of food intake in the domestic chicken and its interaction with gastrointestinal control, *British Poultry Science*, 22:333–346.
- Siragusa, G. R., Haas, G. J., Matthews, P. D., Smith R. J., Buhr, R. J., Dale, N. M. and Wise, M. G. 2008.** Antimicrobial activity of lupulone against *Clostridium perfringens* in the chicken intestinal tract jejunum and caecum. *Journal of Antimicrobial Chemotherapy*, 61: 853–858.
- Songer, J. G. 1996.** Clostridial enteritic diseases of domestic animals. *Clinical Microbiology Review*. 9: 216-234.
- Svobodova I., Steinhäuserová, I. Nebola, M. 2007.** Incidence of *Clostridium perfringens* in Broiler Chicken in the Czech Republic. *Acta. Vet. Brno*. 76: S25-S30.
- Taormina, P. J, Niemira B. A, and Berchat , L. R 2001.** Inhibitory activity of honey against food borne pathogens as influence by the presence of hydrogen peroxide and level of antioxidant power, *Journal of food microbial*, 69:217-225.
- Voidarov, C., Alexopoulos, A., Plessas, S., Karapanov, A., Mantzourani, I., Stavropoulov, E., Fotou, K., Tzora, A., Skoufos, I. and Bezirtzoglov, E. 2011.** Antibacterial activity of different honeys against pathogenic bacteria. *Anaerobic*, 17(6):375-379
- Yoo, H. S., Lee, S. U., and Park, K. Y. 1997.** Molecular typing and epidemiology survey of prevalence of *Clostridium perfringens* types by multiplex PCR. *Journal of Clinical Microbiology*, 35: 228-232.

Received: 25th August, 2016

Accepted: 12th March, 2017