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Effect of sun dried whole bulb garlic powder on nutrient utilization, blood parameters, duodenum morphology and faecal microbial load in broiler chickens

JASWINDER SINGH[1], MANJINDER SHARMA[2], NITINDEV SINGH[3], PAVITER KAUR[4], A P S SETHI[5] and S S SIKKA[6]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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ABSTRACT

This experiment was conducted to study the effect of sun dried whole bulb garlic powder (GP) as phytogenic alternative to antibiotic growth promoters in broilers. Day-old broiler chicks (210) were distributed to five treatments with three replicates. Dietary treatments comprised of basal diet as control (C), antibiotic group receiving 0.1g/kg oxytetracycline (AB), 1.0, 1.5 and 2.0% of garlic powder (GP) added to basal diet as GPL (low), GPM (medium) and GPH (high). A metabolism trail was conducted at the end of fifth week to study nutrient utilization pattern. Blood sample of one bird/replicate on 35th day was collected from jugular vein. Small representative pieces of duodenum were collected from each sacrificed bird to study structural changes in the duodenum morphology. Fresh faecal material was collected and same was used to study viable bacterial cell counting. Results revealed that inclusion of GP did not significantly effected the dry matter metabolizability, ether extract digestibility, per cent nitrogen and phosphorus retention. Higher level of garlic supplementation significantly lowered the serum glucose, triglyceride and cholesterol level. Largest villus height in duodenum and improved villus height/crypt depth ratio were observed in 1.5% GP supplemented group. Total bacterial count in 1.5 and 2.0% GP supplemented groups and *E. coli* count in all the garlic supplemented treatments were statistically comparable with antibiotic fed group. It can be concluded that 1.5% sun dried GP can be used as alternative to antibiotic growth promoters in broilers as well as for producing designer chicken for the consumers.

Key words: Blood parameters, Broiler, Duodenum morphology, Garlic powder, Nutrient utilization

The consistent use of antimicrobial growth promoters (AGPs) at sub-therapeutic level in the poultry industry owing to various well quoted benefits can result in the development of drug resistant bacteria (Alexander *et al.* 2008), which poses a serious threat to human life. Realising the sensitivity of the issue, countries like European Union had banned the use of antibiotic as AGPs in animal production. Plants and plant derived products have multiple effects on animal including appetite stimulator, enhance digestive secretion, immuno-stimulant, bactericidal, antiviral, antioxidants and are termed as phytogenics (Hashemi and Davoodi 2011). Garlic (*Allium sativum*) - the wonder drug of the herbal world due to its multi functional benefits. Garlic traditionally known as "Lasun" possess at least 33 sulphur containing compounds, several enzymes, amino acid and minerals (Newall *et al.* 1996). The major active ingredients in garlic are allicin, ajoene, diakyl polysulphides, s-allylcysteineetc which may be responsible for the various properties of garlic (Canogullari *et al.* 2010). *In vitro* studies have shown that garlic possess antibacterial, antifungal, antiparasitic, antiviral (Ankri and Mirelman 1999) and antioxidant (Prasad *et al.* 2009) properties. Earlier, Singh *et al.* (2015) discussed the effect of whole bulb garlic powder on growth performance, carcass quality and meat quality parameters in broilers. In the present study, effect of garlic powder on nutrient utilization, blood parameters, duodenum morphology and faecal bacterial load are elaborated.

Present address: [1]DES - Veterinary Science (jaswindervet@rediffmail.com), Department of Veterinary and Animal Husbandry Extension Education, [2]Assistant Professor (mannsharma77@gmail.com), Department of Veterinary Physiology, [3]Assistant Professor (drndsingh@gmail.com), Department of Veterinary Pathology, [4]Assistant Professor (paviterkaur@rediffmail.com), Department of Veterinary Microbiology, [5]Senior Nutritionist (apss_pau_ldh@yahoo.co.in), [6]Senior Nutritionist (sssikka04@rediffmail.com), Department of Animal Nutrition.

MATERIALS AND METHODS

Day-old broilers chicks (210) were randomly assigned to five different dietary treatments. Each dietary treatment had three replicates each having seven male and seven female broiler birds. The feeding was done in two phases i.e. starter (0–21 days) and finisher (22–35 days) phase as per BIS (1992). Ingredients and chemical composition of basal diets are presented in Table 1. Diet C served as control while diet AB was supplemented with antibiotic (oxytetracycline) @ 0.1g/kg as growth promoter. Garlic

Table 1. Ingredients and chemical composition of basal (starter and finisher) diets

Ingredient	Starter (S)-0-21 days	Finisher (F)-22-35 days
Maize	55.64	60.79
Soybean meal	38.10	32.14
Oil	2.0	3.0
Di-Calcium phosphate	1.7	1.5
Limestone powder	1.5	1.6
Additives*	+	+
Metabolizable energy (Kcal/kg)	2900	3021
Chemical composition		
Crude protein	21.42	19.14
Crude fat	3.03	5.7
Crude fibre	5.43	4.33
Ash	6.8	5.5
Acid insoluble ash	0.47	0.56
Calcium	1.16	1.18
Av Phosphorus	0.49	0.48

*Additives: Each 100 kg ration contained Vitamin A, 8,25,000 IU; Vitamin D_3, 1,20,000 IU; Riboflavin, 500 mg; Vitamin K, 100 mg; Vitamin E, 800 mg; Thiamin, 80 mg; Pyridoxine, 160 mg; Cynacobalamin, 800 mg; Niacin, 1,200 mg; Calcium pantothenate, 800 mg; Manganese sulphate, 25 g; Zinc sulphate, 25 g; Ferrous sulphate, 10 g; Copper sulphate, 500 mg; Potassium iodide, 100 mg; Enzymes, 20 g; Lysine, 0.070 g (S) 0.010 g(F); Methionine 0.170 g (S) 0.100 g (F); Coccidiostat, 100 g; Toxin binder, 100 g; Salt, 370 g.

bulbs were procured from Punjab Agricultural University, Ludhiana and bulbs were fragmented into pieces and paste was prepared, which was then sun dried and grounded to obtain whole bulb garlic powder (GP). Diets GPL, GPM and GPH were supplemented with this GP @ 1.0 (low), 1.5 (medium) and 2.0% (high) level replacing maize in the ration respectively. All the experimental diets and herbs were analysed for proximate principles, phosphorus (AOAC 2000) and calcium (Talpatra *et al.* 1940) content.

Nutritional composition of this GP includes 91.22% dry matter, 13.69% crude protein, 1.63% ether extract, 10.82% crude fibre, 0.2% calcium, 0.43% phosphorus, 4.376 ppm copper, 32.09 ppm zinc, 188.5 ppm iron, 1.414 ppm manganese and 3,615.30 kcal/kg gross energy. Weighed amount of respective diet was offered daily to all groups to ensure *ad lib.* feeding. Fresh clean water was made available to the birds throughout the experimental period. The birds were reared on deep litter system maintaining standard managemental practices. Animal ethic committee approval was taken for conducting the study. A metabolism trail was conducted at the end of 5th week using total collection method. Two birds (one male and one female) from each replicate of comparable body weight were selected and shifted in cage. The birds were housed in cages for a period of five days for acclimatization. During this period, weighed amount of respective feed was offered to the birds twice daily. After five days of adaptation period, the feed residue was removed. Weighed amount of fresh feed was offered

Table 2. Effect of GP supplementation on per cent nutrient utilization in broiler chicks

Parameter (%)	C	AB	GPL	GPM	GPH	SEM
DM	78.02	79.06	80.24	77.85	78.83	0.478
Nitrogen	70.22	71.92	75.38	75.19	74.78	0.854
EE	85.91	88.43	85.67	88.89	88.57	0.626
CF	22.38^{a}	22.03^{a}	23.55^{a}	34.99^{ab}	41.23^{b}	2.553
Ca	43.17^{a}	52.52^{b}	41.92^{a}	44.05^{a}	44.07^{a}	1.269
P	43.85	51.27	40.76	45.47	46.28	1.764

^{a,b,c}Means bearing different superscripts in a row differ significantly (P<0.05).

Table 3. Effect of GP supplementation on blood parameters in broilers

Parameter	C	AB	GPL	GPM	GPH	SEM
Hb (gm%)	8.93	9.53	10.07	10.00	9.07	0.215
PCV (%)	28.33	29.50	28.84	30.83	28.34	0.611
GLU (mg/dl)	236.67^{bc}	259.67^{c}	230.66^{bc}	196.00^{ab}	173.00^{a}	9.994
TG (mg/dl)	106.02^{b}	99.08^{ab}	104.02^{b}	94.10^{ab}	90.55^{a}	2.066
CHOL (mg/dl)	133.33^{b}	124.0^{ab}	127.67^{ab}	124.00^{ab}	102.00^{a}	4.089
TP (g/dl)	3.93	3.90	3.80	3.90	4.00	0.047
ALB (g/dl)	1.83	1.77	1.70	1.87	1.60	0.048

^{a,b,c}Means bearing different superscripts in a row differ significantly (P<0.05).

to each group, for three consecutive days. Fresh water supply and 24 h light were assured during the metabolism trail. The feed residue was removed on the fourth day and weighed back to record the actual feed consumption for each group. Faeces voided by each replicate was collected daily, weighed and dried separately at 80°C in hot air oven. Ten millilitres of 10% sulphuric acid was mixed with collected faeces to check nitrogen loss. Dried faeces of each group for three consecutive days were pooled and grounded for chemical analysis.

Blood sample of one bird/replicate totaling to three birds from each treatment on 35th day were collected from jugular vein in two EDTA and non-EDTA containing vials. The serum was prepared from the later. EDTA containing sample was used for determining haemoglobin (haemometer method) and packed cell volume, while serum was used for estimating glucose, triglycerides, cholesterol, total protein and albumin content. The measurements were taken using commercial kits.

After thorough gross examination, small representative pieces (approximately 0.5 cm length) of duodenum were collected from each sacrificed bird and fixed in 10% neutral buffered formalin. After proper fixation for 3–4 days, tissues

were cut into thinner sections (1–2 mm thick). The tissues were washed in running water for 7– 8 h, dehydrated in ascending grades of ethyl alcohol, cleared in benzene and embedded in paraffin wax (melting point 58°C). The paraffin blocks were prepared and the sections were cut at 4–5 µ thickness with a hand operated microtome. The paraffin embedded sections were then passed through sequential steps of deparaffinisation in xylene, rehydration through descending grades of ethyl alcohol to running water. Sections were stained by routine haematoxylin and eosin stain.

Fresh faecal material was collected and same was sent to Department of Veterinary Microbiology, GADVASU. Viable bacterial cell counting was carried out using Miles-Misra technique. Serial tenfold dilution of the faecal samples containing bacteria were carried out as accurately as possible to minimize avoidable errors and an aseptic technique was used. Ranges of dilution were used and an inoculum of 0.02 ml, delivered as a drop was placed on the agar (Brain heart infusion agar for total bacterial load and Hichrome *E. coli* agar for *E. coli).* At least 4 drops/sample dilution were used. The inoculums were allowed to dry and the plates were incubated at 25–37°C for 24–48 h. Colony count obtained from the sample inoculums were then used to determine the number of bacteria/ml of original sample. Data were subjected to analysis of variance using SPSS (version 18) and treatments mean were compared using Duncan's multiple range tests at 95% significance level.

RESULTS AND DISCUSSION

Dry matter metabolizability, ether extract digestibility, per cent nitrogen and phosphorus retention remained unaffected due to dietary treatments (Table 2). Crude fibre digestibility increased as the GP level increased and was highest (P<0.05) in GPH group followed by GPM. The crude fibre digestibility in GPL, AB and C did not differ significantly (P<0.05). Significantly (P<0.05) highest calcium retention was observed in antibiotic fed group (AB), but per cent calcium retention in all other treatment was found to be almost similar. Increased digestive secretion (Puvaca *et al.* 2013), enhancement of intestinal activities of trypsin, lipase and amylase (Lee *et al.* 2004) and improved gut morphological characteristics (Jamroz *et al.* 2006) are the major mechanisms through which phytoadditives exerts their beneficial effect on the nutrient digestibility.

Significant (P<0.05) effect of dietary treatments was observed on glucose, triglycerides (TG) and cholesterol level (Table 3). Supplementation of GP at 2.0% level (GPH) significantly (P<0.05) lowered the serum glucose value as compared to control and antibiotic fed groups. Highest glucose level (P<0.05) was recorded in AB and was comparable with glucose level found in C and GPL groups. Similarly, inclusion of GP at 2.0% (GPH) level significantly (P<0.05) reduced the TG and cholesterol level as compared to control. The present results are in agreement with Issa and Omar (2012), who reported that supplementation of garlic powder at 0.2 and 0.4% significantly reduced the cholesterol, triglycerides, low density lipoprotein and increased the high density lipoprotein level in Cobb broiler blood as compared to control birds.

Khan *et al.* (2012) also reviewed the anti-cholesteremic effect of garlic and reported that hypocholesterolaemic and hypolipidemic action of garlic products is through depressing the activities of liver enzymes such as malic acid, fatty acid synthase, glucose-6-phosphatase dehydrogenase and 3-hydroxy-3-methyl-glutaryl-CoA reductase (Qureshi *et al.* 1983, Mahmoud *et al.* 2010). Hypoglycaemic effect of garlic was due to improvement in pancreatic secretion of insulin from beta-cells or its release from bound insulin (Jain and Vyas 1975). However, Banerjee and Maulik (2002) revealed that allyl propyl disulphide-one of the active ingredients of garlic, may lower glucose by competing with insulin for insulin inactivating sites in the liver, which ultimately results in increased free insulin.

Structure of intestinal mucosa reflects the health condition of intestine. The significantly largest (P<0.05) villus height in duodenum was observed in 1.5% GP supplemented group (GPM) as compared to villus height in C, AB and GPL groups (Table 4).

Supplementation of GP at 1.5% level (GPM) significantly (P<0.05) improved the villus height:crypt depth ratio compared to all other treatments. Total bacterial

Table 4. Effect of GP supplementation on duodenum morphology and faecal bacterial count in broilers

Parameter	C	AB	GPL	GPM	GPH	SEM
Villus height (µm)	1412.51^{a}	1419.80^{a}	1394.17^{a}	2035.45^{b}	1570.62^{ab}	89.240
Crypt depth (µm)	95.15	87.24	99.27	75.51	91.42	5.141
Villus height: crypt depth ratio	14.84^{a}	16.28^{a}	14.05^{a}	26.95^{b}	17.18^{a}	1.478
Total bacterial (10^{10}) organisms/ml of sample	12.0^{c}	2.0^{a}	6.50^{b}	4.0^{ab}	3.3^{ab}	1.228
E. coli (10^{8}) organism/ml of sample	8.50^{b}	1.0^{a}	6.3^{ab}	1.1^{a}	1.5^{a}	1.152

^{a,b,c}Means bearing different superscripts in a row differ significantly (P<0.05)

and *E. coli* count/ml of faecal sample were significantly (P<0.05) higher in C compared to rest of the treatments (Table 4). Lowest total bacterial and *E. coli* count was observed in antibiotic group (AB). Total bacterial count in GPM and GPH groups and *E. coli* count in all the garlic supplemented treatments were statistically comparable with antibiotic fed group (AB).

Xu *et al.* (2003) revealed that higher the length of villus, more will be the surface area for absorption of nutrients. Deeper crypt indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins (Yason *et al.* 1987). Reduced villus height:crypt depth ratio can also indicate presence of toxin, reduced absorption of nutrients, increased secretion in gastrointestinal tract, diarrhoea, reduced disease resistance and lower overall performance. Results of present study were in agreement with Saeid *et al*. (2013), who also reported the improved intestinal morphological characteristics like villi length and small crypts in birds receiving 0.5% garlic powder containing diet as compared to control.

The results of present study revealed that garlic has the potential to act as phytobiotic growth promoter in the broiler chicken. It could be concluded that 1.5% sun dried GP supplemented group had better performance during both starter and overall period than the control and had significantly higher benefit:cost ratio than all other treatments (Singh *et al.* 2015). GP improved the nutrient utilization by improving the duodenum morphology as evident from improvement in villus:crypt ratio and reduction in the faecal bacterial load. Sun dried GP @ 1.5% is an easy and economical alternative to antibiotic growth promoters besides helping in production of consumer oriented meat.

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REFERENCES

Alexander T W, Yanke L J, Topp E, Olson M E, Read R R, Morck D W and Mcallister T A. 2008. Effect of sub-therapeutic administration of antibiotics on the prevalence of antibiotic resistant *Escherichia coli* bacteria in feedlot cattle. *Applied Environmental Microbiology* **74**: 4406–16.

Ankari S and Mirelman D. 1999. Antimicrobial properties of allicin from garlic. *Microbial Infection* **2**: 125–29.

AOAC. 2000. Official Methods of Analysis, 16th edn. Association of Official Analytical Chemist, Washington, DC.

BIS. 1992. Requirement for chicken feed. IS:1374–1992, Manak Bhavan, 9 Bahadurshah Zafar Marg, New Delhi-110 001.

Banerjee S K and Maulik S K. 2002. Effect of garlic in cardiovascular disorder: a review. *Nutrition Journal* **1**: 4–18.

Canogullari S, Baylan M, Erdogan Z, Duzguner V and Kucukgul A. 2010. The effect of dietary garlic powder on performance, egg yolk and serum cholesterol concentration in laying quails. *Czech Journal of Animal Science* **55**: 286–93.

Hashemi S R and Davoodi H. 2011. Herbal plants and their derivatives as growth and health promoters in animal nutrition. *Veterinary Research Communication* **35**: 169–80.

Jain R C and Vyas C R. 1975. Garlic in alloxan induced diabetic rabbits. *American Journal of Clinical Nutrition* **28**: 684–85.

Jamroz D, Orda J, Kamel C, Wiliczkiewicz A, Wertelecki T and Skorupinska J. 2006. The influence of phytogenetic extracts on performance, nutrient digestibility, carcass characteristics and gut microbial status in broiler chickens. *Journal of Animal Feed Science* **12**: 583–96.

Khan R U, Zikousefat Z, Tufarelli V, Naz S, Javdani M and Laudadio V. 2012. Garlic (*Allium sativum*) supplementation in poultry diets: effect on production and physiology. *World's Poultry Science Journal* **68**: 417–24.

Lee K W, Everts H, Kappert H J, Van J, Lemmenj A G, Frehner M and Beynen A C. 2004. Growth performance, intestinal viscosity, fat digestibility and plasma cholesterol in broiler chickens fed a rye containing diet without or with essential oil components. *Poultry Science* **9**: 613–18.

Mahmoud K Z, Saad M, Gharaibeh H, Zakaria A and Amer M. 2010. Garlic (*Allium sativum*) supplementation: Influence on egg production, quality and egg cholesterol level in layer hens. *Asian Australasian Journal of Animal Science* **23**: 1503–09.

Newall C A, Anderson L A and Phillipson J D. 1996. Herbal medicine: a guide for health care professionals. Pharmaceutical press, London.

Prasad R, Rose M K, Virmani M, Garg S L and Puri J P. 2009. Lipid profile of chicken (*Gallus domesticus*) in response to dietary supplementation of garlic. *International Journal of Poultry Science* **8**: 270–76.

Puvaca N, Stanacev V, Glamocic D, Levic J, Peric L, Stanacev V and Mili D. 2013. Beneficial effect of phyto additives in broilers nutrition. *World Poultry Science Journal* **69**: 27–34.

Qureshi A A, Din Z Z, Abuirmeileh N, Burger W C, Ahmad Y and Elson C E. 1983. Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: Impact on serum lipid. *Journal of Nutrition* **113**: 1746–55.

Saeid J M, Mohamed A B and AL-Baddy M A. 2013. Effect of adding garlic powder (*Allium sativum*) and black seed (*Nigella sativa*) in feed on broiler growth performance and intestinal wall structure. *Journal of Natural Sciences Research* **3**(1): 35–41.

Singh J, Sethi A P S, Sikka S S, Chateli M K and Kumar P. 2015. Effect of sun dried whole bulb garlic powder on growth, carcass characteristics and meat quality of commercial broilers. *Indian Journal of Animal Sciences* **85**: 67–71.

Talapatra S K, Roy S C and Sen K C. 1940. Estimation of phosphorus, chlorine, calcium, sodium and potassium in food stuffs. *Indian Journal of Veterinary Science and Animal Husbandry* **10**: 243–58.

Toghyani M, Toghyani M, Gheisari A, Ghalamkari G and Eghbalsaied S. 2011. Evaluation of cinnamon and garlic as antibiotic growth promoters substitutions on performance, immune responses, serum biochemical and haematological parameters in broilers chicks. *Livestock Science* **38**: 167–73.

Xu Z R, Hu C H, Xia M S, Zhan X A and Wang M Q. 2003. Effect of dietary fructooligosaccharide on digestive enzymes activities, intestinal microflora and morphology of male broilers. *Poultry Science* **82**: 648–54.

Yason C V, Summers B A and Schat K A. 1987. Pathogens of rotavirus infection in various age groups of chickens and turkey: pathology. *American Journal of Veterinary Research* **6**: 927–38.