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Influence of herbal mixture, exogenous enzymes and butyric acid supplementation on nutrient digestibility, carcass characteristics and economics of broiler chickens

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Abstract
Three hundred commercial straight run, day old Ven Cobb 400Y chicks were divided into six groups with 5 replicates of 10 birds in each to assess the efficacy of herbal mixture, exogenous enzymes and butyric acid on their performance. The chicks were placed on basal diet with no supplement in negative control (NC) and basal diet supplemented with antibiotics, alcoholic extract of 0.5% herbal mixture, 0.5% herbal mixture (alcoholic extract) + 0.1% butyric acid, 0.5% herbal mixture (alcoholic extract) + 0.03% exogenous enzymes and combination of 0.5% herbal mixture (alcoholic extract) + 0.03% exogenous enzymes + 0.1% butyric acid in PC, HE, HEB, HEE and HEBE groups, respectively. The results of the analyzed data showed that DM and CP digestibility coefficient of herbal based non-antibiotic feed additive groups were similar to NC and PC group birds. Drawn weight yields were not affected by any additive supplements. The abdominal fat was significantly (P<0.005) lower in HEB and HE group birds as compare to NC and PC groups. Profit/bird live weight, benefit cost ratio and profitability of HEB group was significantly (P<0.05) better than NC, but (P<0.05) lower to PC group. It was concluded that, supplementation of alcoholic extract of 0.5% herbal mixture along with 0.1% butyric acid had no adverse effect on nutrient digestibility and carcass characteristics; however, profitability was higher than negative control birds.

Keywords: Herbal mixture, butyric acid, nutrient digestibility, carcass characteristics and economics

Introduction
Now a days poultry sector is one of the most popular and fastest growing livestock sector in India, contributing 10% of livestock and 0.7% of total GDP. The demand for low cost and processed quality chicken meat is growing by 15-20% annually. The aim of poultry production is to get proper growth rate, better feed conversion efficiency and maintaining optimal health to maximize production in terms of meat and egg. In poultry diet, antibiotic growth promoters (AGPs) are used for the protection against some diseases, toxins, improvement in nutrients absorption in intestines etc. However, the efficacy of antibiotics reduced due to development of resistance in pathogenic bacteria; besides this residues of antibiotics in meat have negative impact on public health. In most of the countries AGPs has been either regulated or banned (Lesson *et al*., 2015) [14]. So, there is need of finding alternatives to growth-promoting antimicrobials without affecting their production. Several substances like essential oil of herbs, organic acids, probiotics, prebiotics, exogenous enzymes etc. have been investigated in recent years, which are able to support productive performance and prevent the incidence of some diseases in poultry (Huyghebaert *et al*., 2011) [10].
Phytogenic feed additives are plant derivatives of plant extraxts, herbs and spices with extended activities viz., endogenous secretions in the gut, stimulation of feed intake and better growth. Herbs like aloe vera (*Aloe barbadensis*), ashwagandha (*Withaniasomnifera*), mangrail (*Nigella sativa*), turmeric (*Curcuma longa*), methi/fenugreek (*Trigonellafoenum-gracium*), neem (*Azadiractaindica*), punarnava (*Bhoeraviadiffusa*), tulsi (Ocimum sanctum), bhuiamla (*Phyllanthus emblica*) and garlic (*Allium sativa*) are commonly used as medicinal herbs in India and have antibacterial, antifungal, anti-inflammatory, antioxidant, growth promoting, hepatoprotective and immuno-stimulatory activities. Non starch polysaccharides (NSP) present in corn and soybeans are poorly digested by monogastric animals; represent a potential source

of nutrients. However, NSP are capable of binding nutrients and complex formation with the enzymes of digestive system and few regulatory proteins of gastro-intestinal system, (Angkanaporn *et al*., 1994) [4] making the nutrients unavailable. Combinations of feed enzymes such as xylanase, amylase and protease improve the digestibility of diets and performance of broiler. Organic acids have diversified useful effects on micro-flora and function of gut, inhibits pathogenic bacteria, improve mineral absorption, preserve feed from microbial invasion and stimulate recovery from cage fatigue (Golshadi *et al*., 2015) [8]. It can improve nutrient utilization in poultry diets (Kim *et al*., 2011) [13], enhance phytate-P utilization (Liem *et al*., 2008) [15], reduce the colonization of pathogenic micro-organisms on intestinal wall (Ricke *et al*., 2003) [20], synthesis of toxins produced by the bacteria and aflatoxin content of feed (Mendez Albores *et al*., 2007) [16]. Few research studies are reported regarding the use of alcoholic extract of herbal mixture, butyric acid and exogenous enzymes on broilers, but research studies using the combinations of alcoholic extract of herbal mixture with butyric acid and/or exogenous enzymes are lacking. Therefore, the present experiment was conducted to investigate the efficacy of an alcoholic extract of herbal mixture with butyric acid and/or exogenous enzymes on nutrient utilization, carcass characteristics and economics of broiler chickens.

Materials and Methods

The forty two days trial was conducted at the Livestock Farm Complex, College of Veterinary Sciences and Animal Husbandry, Acharya Narendra Dev University of Agriculture and Technology (ANDUAT), Kumarganj, Ayodhya (U.P.) to evaluate the effect of herbal mixture, exogenous enzymes and butyric acid supplementation on the performance of broiler chickens. Three hundred day old Ven-Cobb 400Y straight run chicks were randomly divide into 6 groups with 5 replicates of ten chicks in each. The chicks were placed on basal diet without supplement in negative control (NC) and basal diet with supplementation of antibiotics, alcoholic extract of 0.5% herbal mixture, 0.5% herbal mixture (alcoholic extract) + 0.1% butyric acid, 0.5% herbal mixture (alcoholic extract) + 0.03% exogenous enzymes and combination of 0.5% herbal mixture (alcoholic extract) + 0.03% exogenous enzymes + 0.1% butyric acid in PC, HE, HEB, HEE and HEBE groups, respectively. All the broiler chicks were fed *adlibitum*, according to three phase feeding programme with a pre-starter, starter and finisher ration from day 1-7, 8-21 and 22 to 42 days of age, respectively (BIS, 2007) [11]. A polyherbal mixture was prepared by using turmeric rhizomes, mangrail seeds, methi seeds, neem leaves, tulsi leaves, aloe vera leaves, garlic bulbs, punarnava roots, ashwagandha roots and bhuiamala roots in a certain proportion and extracted with alcohol. Then premix was added successively in the whole lot. The chicks were kept hygienically on floor litter system in separate pens under standard managemental conditions. The birds were regularly observed for stress, disease or different behavior from normal one. The proximate and ingredient composition of broiler prestarter, starter and finisher rations are presented in Tables 1 and 2, respectively.

Table 1: Ingredient composition (%) of broiler prestarter, starter and finisher diets

Ingredient (kg)	Pre-starter	Starter	Finisher
Maize	50.42	55.52	59.82
Soybean meal	42.00	36.40	27.40
Rice polish	-	-	5.00
Vegetable fat	4.48	5.18	5.43
Dicalcium phosphate	1.15	0.97	0.80
Limestone powder	0.90	1.04	0.75
Common salt	0.40	0.38	0.36
DL-Methionine	0.25	0.18	0.16
Lysine	0.15	0.08	0.03
Choline chloride	0.10	0.10	0.10
Vitamin premix	0.05	0.05	0.05
Mineral premix	0.10	0.10	0.10

*****Supplies per kg diet: Vitamin A, 16500IU; Vitamin D_3, 3200IU; Vitamin E, 12mg; Vitamin K, 2 mg; VitaminB_2, 10mg; Vitamin B_6, 2.4 mg ; Vitamin B_{12},12µg; Niacin, 18 mg; Pantothenic acid, 12 mg; Mn, 90mg ; Zn, 72mg; Fe, 60mg; Cu, 10 mg; I, 1.2 mg.

A metabolism trial of seven days duration was conducted in last week of trial period to determine the retention of nutrients. The proximate analysis of feeds, faeces and residues were performed as per the method of AOAC, 2000 (William, 2000) [25].

Table 2: Nutrient composition of broiler pre-starter, starter and finisher ration

Nutrients	Pre-Starter	Starter	Finisher
Dry Matter (%)	87.15	87.77	89.22
Crude protein (%)	23.14	22.09	20.31
Crude fiber (%)	3.05	3.25	3.45
Ether extract (%)	4.98	5.80	7.78
Total ash (%)	5.79	5.18	6.86
Metabolizable energy* (Kcal/kg)	3003.10	3107.00	3203.40

*Calculated value

At the end of feeding trial i.e. on 42th day, one bird from each replicate (Five birds /group) was randomly selected and slaughtered for carcass trait studies. Prior to slaughter birds were off fed for 12 hours. After slaughtering and dressing, the warm carcass weights were recorded. The dressed carcasses were separated into different parts; legs, breast, back, wings and neck and weight of each cut was recorded. Edible cuts consisted of skin with subcutaneous fat, lean meat and giblet (gizzard, heart and liver). Processing losses was calculated by weighing the inedible offals comprised of slaughter offal (blood, feathers, head, feet, gastrointestinal tract with the digesta and peri-intestinal fat), other offal (trachea, lungs, kidneys, reproductive organs) and abdominal fat. The relative weight of dressed, giblet, drawn, spleen, pancreas and processing losses were calculated as percentage of live weight whereas different edible cuts weight were calculated as percentage of carcass weight.

The economics of broiler rearing under different dietary treatments was calculated cost index percent, profit/bird, return on feed, feed price ratio and economic efficiency at the end of sixth week. The cost of production included the cost of chick and cost of feed consumed. The cost of feed included the cost of feed ingredients, antibiotics and herbals. The prices of all feed ingredients including feed additives were considered as per the rate list supplied by respective manufacturers or suppliers. Due to equality in the fixed costs across all dietary treatments they were not included in the analysis.

All the data generated was statistically analyzed by using SPSS, version-20 software. The obtained data was subjected to analysis of variance (ANOVA) and means were compared by using Duncan's multiples range test.

Results and Discussion

Digestibility of nutrients

The influence of antibiotic or herbal based non-antibiotic feed additive on nutrient digestibility of broilers in different groups is presented in Table 3. The results revealed that the apparent digestibility coefficients of dry matter (DM), crude protein (CP), ether extract (EE) and crude fiber (CF) were similar (P>0.05) in comparison to NC and PC group birds. It was also evident that among the non-antibiotic supplemented groups, the digestibility coefficient (%) of DM, CP and CF were non significantly improved in HEBE group birds than NC group birds.

Table 3: Apparent nutrient digestibility (%) of broilers supplemented with alcoholic extract of an herbal mixture, exogenous enzyme or butyric acid in the diet

Attributes	NC	PC	HE	HEB	HEE	HEBE	SEM	P-value
Dry matter (DM)	68.38	73.69	70.54	70.41	68.96	71.49	0.666	0.238
Ether extract (EE)	96.66	97.71	96.38	96.08	96.51	97.42	0.210	0.173
Crude protein(CP)	69.31	72.70	71.21	70.15	70.13	71.02	0.393	0.179
Crude fiber (CF)	18.73	19.25	19.83	19.57	20.06	21.31	0.452	0.723

Means with different small letter superscripts between groups differ significantly (P<0.05).

Similarly the supplementation of phytobiotics and enzymes blend improved nutrient digestibility in Ven-Cobb 400Y broilers (Singh, 2018) [22]. Inclusion of 0.2 g/kg organic acid in diet of broilers significantly (P<0.05) improved the crude fibre digestibility (Rodjan *et al*., 2017) [21]. In another study the crude fat digestibility was better on supplementation of coated butyric acid at a rate of 0.2, 0.3, or 0.4 g/kg in the broiler diet (Kackmarek *et al*., 2016) [12]. Xylanase supplementation, non significantly (P>0.05) improved the ileal digestibility of protein and energy by 3% and 6%, respectively (Nian *et al*., 2011) [18]. In this study, the mechanism by which phytobiotics, enzymes and organic acids stimulate digestion process is probably due to the stimulation of pancreatic digestive enzymes and bile secretions. Organic acids and exogenous enzymes may weaken the structure of crude fiber, thus making gastric juices able to penetrate more easily into the cell wall. On the other hand, as a herbal product contain phyto-chemicals that enhance the secretion and activities of terminal digestive enzymes of small intestinal mucosa (Ramakrishna *et al*., 2003) [19] and improve the digestibility of nutrients. Beside this, organic acids also reduce the gut pH that helps in the digestion of micronutrients.

Carcass parameters

The carcass characteristics of broilers presented in Table 4 revealed that the giblet weight of non-antibiotic group birds were similar to NC and significantly (P<0.05) higher than PC group birds. Spleen weight was ranged from 0.10 to 0.13 percent of live weight and did not showed any effect of treatments. Weights of pancreas of HEB, HEE and HEBE group birds were similar to NC and PC groups and significantly (P<0.05) lower than HE group birds. Dressed weight of all the treatment groups were similar and significantly (P<0.05) lower than NC group birds. The eviscerated and drawn yields were not affected due to any additive supplements. Viscera weight of HEE and HEBE groups were significantly (P<0.05) lower than NC group birds. Abdominal fat contents in all supplemented groups were lesser than NC group birds.

Table 4: Carcass characteristics (% of live BW) of broiler chickens supplemented with alcoholic extract of an herbal mixture, exogenous enzyme or butyric acid in the diet

Attributes	NC	PC	HE	HEB	HEE	HEBE	SEM	P-value
Giblet weight	4.10^{a}	3.61^{b}	4.315^{a}	4.33^{a}	4.06^{a}	4.12^{a}	0.066	0.006
Spleen weight	0.10	0.12	0.11	0.13	0.13	0.13	0.004	0.338
Pancreas weight	0.14^{bc}	0.14^{bc}	0.18^{a}	0.16^{b}	0.13^{c}	0.15^{b}	0.004	<0.001
Dressed weight	90.27^{a}	87.24^{b}	88.06^{b}	87.55^{b}	87.53^{b}	86.62^{b}	0.317	0.005
Eviscerated weight	75.54	76.28	76.25	75.72	76.44	76.75	0.174	0.358
Drawn yield	79.64	79.89	80.56	80.05	80.51	80.88	0.177	0.333
Viscera weight	8.84^{a}	6.08^{b}	6.48^{ab}	6.63^{ab}	6.02^{b}	4.46^{b}	0.385	0.023
Abdominal fat	1.79^{a}	1.26^{b}	1.01^{c}	0.87^{c}	1.00^{c}	1.28^{b}	0.068	<0.001
Inedible component weight	20.36	20.11	19.43	19.95	19.48	19.12	0.177	0.333

Means with different small letter superscripts between groups differ significantly (P<0.05).

Similar to the present findings, supplementation of herbal mixtures did not show any significant differences among groups on drawn yields (Singh *et al.*, 2016) [23]. In another study inclusion of a mixture of turmeric, mangrail and amla in the diet of broiler chickens had no effect on carcass characteristics (Chaudhary *et al.*, 2014) [6]. The supplementation of enzymes had any significant effect on carcass traits of birds (Sonu *et al.*, 2018) [24]. Contrary to the present findings, higher carcass yield was found on supplementation of enzyme complex in broiler chickens (Andrade *et al.*, 2018) [3]. Supplementation of cinnamon oil and sodium butyrate or by antibiotic in the diet broilers had

no effect on carcass characteristics i.e giblet weight (heart, liver and gizzard), eviscerated weight and abdominal fat percentage. (Gomathi *et al.*, 2018) [9]. However, in another study it was found that the organic acid supplementation upto the level of 0.2 % in the diet of broilers significantly (P<0.05) improved the carcass weight (Mohamed *et al.*, 2018) [17].

Economics

The economics of broiler production are presented in Table 5. Total expenditure to rear an individual bird in different groups was ranged between Rs. 196.77 and Rs. 217.44, and exhibited that cost/bird was higher in HE and HEBE groups birds than antibiotic or without any additives supplemented group birds. The cost index percent of HEB, HEE and HEBE groups were significantly (P<0.05) lower than HE and NC, however higher to PC groups. The profit per bird and return on feed was significantly (P<0.05) higher in PC group followed by HEB, HEBE, HE and HEE as compare to NC group birds. The economic efficiency of HE, HEE and HEBE groups were similar to NC and lower to PC group, however HEB groups had higher economic efficiency than NC group.

Table 5: Effect of alcoholic extract of herbal mixture, butyric acid and enzyme supplementation in diets of broiler chickens on relative economics

Attributes	NC	PC	HE	HEB	HEE	HEBE	SEM	P-value
Chick cost/bird (INR)	48.00	48.00	48.00	48.00	48.00	48.00	0.000	-
Feed cost/bird (INR)	143.77^{b}	140.59^{b}	160.06^{a}	142.48^{b}	139.78^{b}	152.99^{ab}	2.185	0.025
Feed additive cost/bird (INR)	0.00^{d}	3.05^{d}	4.38^{c}	5.98^{b}	5.66^{b}	8.43^{a}	0.489	<0.001
Miscellaneous/bird (INR)	5.00	5.00	5.00	5.00	5.00	5.00	0.000	-
Total expenditure/bird (INR)	196.77^{c}	196.64^{c}	217.44^{a}	201.46^{bc}	198.44^{c}	214.42^{ab}	2.350	0.010
Cost index percent	138.58^{a}	102.36^{c}	132.42^{a}	115.99^{b}	117.48^{b}	121.40^{b}	2.493	<0.001
Total income/bird (INR)	207.94^{d}	273.66^{a}	245.47^{b}	245.49^{b}	223.29^{c}	246.11^{b}	4.569	<0.001
Profit/bird (INR)	11.17^{d}	77.02^{a}	28.03^{bc}	44.02^{b}	24.84^{bc}	31.69^{bc}	4.708	<0.001
Return on feed (INR)	40.39^{c}	54.36^{a}	42.03^{bc}	48.12^{b}	47.52^{bc}	43.63^{bc}	0.964	<0.001
Feed price ratio	1.45^{c}	1.94^{a}	1.55^{bc}	1.72^{b}	1.60^{bc}	1.61^{bc}	0.035	<0.001
Economic efficiency	0.06^{c}	0.39^{a}	0.14^{bc}	0.22^{b}	0.12^{bc}	0.15^{bc}	0.028	<0.001

Means with different small letter superscripts between groups differ significantly (P<0.05).

The higher feed cost and total expenditure of broiler production in HE and HEBE group birds might be due to the higher feed intake and additional cost of herbs, exogenous enzymes and organic acids. The higher profit and economic efficiency in alcoholic extract of herbal mixture with butyric acid supplemented (HEB) group are in agreement with the findings of [16],who reported thatdietary inclusion of phytobiotics in the diets was more beneficial in broiler production. Similarly, tulsi leaves extract supplementation as growth stimulant in broilers had more profitablity as compared to the control group (Biswas *et al.*, 2017) [5]. In another study dietary inclusion of xylanase at a rate of 60 g/ton and 100 g/ton improved the net profit per bird (Gade *et al.*, 2017) [7]. Similarly, economic gain in terms of total return and net profit was better in enzymes supplemented broilers (Ahmed *et al.*, 2015) [1]. Dietary supplementation of a combination of herbal extract and organic acids also significantly (P<0.05) improved the economic efficiency and relative economic efficiency in the broilers (Hassan *et al.*, 2015) [2].

Conclusion

It was concluded that supplementation of alcoholic extract of 0.5% herbal mixture along with 0.1% butyric acid had no adverse effect on nutrient digestibility and carcass characteristics. So it may be used as an alternative of antibiotic growth promoters. However, profitability was lower than the conventional system of broiler rearing.

References

1. Ahmed I, Munir S, Jamal MA, Naseer T, Pasha Ditta YA, Mahmud A *et al*. Effect of enzyme complex at different wheat-based diets on growth performance of broilers**.** Journal of Entomology and Zoology Studies. 2015; 5(3):525-531.
2. Hassan HMA, Youssef AW, Ali HM, Mohamed MA. Adding phytogenic material and/or organic acids to broiler diets: effect on performance, nutrient digestibility and net profit. Asian Journal of Poultry Science. 2015; 9(2):97-105.
3. Andrade TS, Nunes RV, Wachholz L, Silva IM, Freitas DM. The effect of exogenous enzymes on the performance and digestibility of nutrients in broiler. Semina: Ciências Agrárias, Londrina. 2018; 39(2):711-718.
4. Angkanaporn K, Choct M, Bryden WL, Annison EF, Annison G. Effects of wheat pentosans on endogenous amino acid losses in chickens. Journal of the Science of Food and Agriculture. 1994; 66(3):399-404.
5. Biswas AK, Rahman MM, Hassan MZ, Sultana S, Rahman MM, Mostofa M. Effect of tulsi (Ocium sanctum) leaves extract as a growth promoter in broiler production. Asian Journal of Medical and Biological Research. 2017; 3(2):226-232.
6. Chaudhary RK, Singh VK, Singh SP, Sahoo B, Singh AK. Comparative efficacy of turmeric (*Curcuma longa*), amla (*Emblica officinalis*) and Mangrail (*Nigella sativa*) as growth promoters in broilers. Animal Nutrition and Feed Technology. 2014; 14**:**273-281.
7. Gade DS, Dhumal MV, Nikam MG, Bhosale D. Influence of different levels of xylanase enzyme on performance, litter quality and economics of broiler chicken International Journal of Agricultural Science and Research. 2017; 7(3):73-82.
8. Golshadi J. Effect of dietary supplementation of butyric acid glycerides on performance, immunological responses, ileal microflora, and nutrient digestibility in laying hens fed different basal diets. Livestock Science. 2015; 178**:**228-236.
9. Gomathi G, Senthilkumar S, Natarajan A, Amutha R, Purushothaman MR. Effect of cinnamon oil and sodium butyrate on growth performance of broilers. Veterinary world. 2018; 11(7):959-964.
10. Huyghebaert G, Ducatelle R, Immerseel FV. An update

on alternatives to antimicrobial growth promoters for broilers. Veterinary Journal. 2011; 187:182-188.
11. IS 1374 (2007). Poultry feeds – specification, (Fifth Revision). Bureau of Indian Standards, 2007.
12. Kackmarek SA, Hejdysz M, Kubis M, Kasprowicz-Potocka M, Rutkowski A. The nutritional value of yellow lupin (*Lupinus luteus L.*) for broilers. Animal Feed Science and Technology. 2016; 222:43-53.
13. Kim DY, Kwon WB, Kim BG. Dietary acidifiers in weanling pig diets: a review. Rev colomb Cien Pecu. 2011a; 24:231-247.
14. Lesson S. Butyrate lancing science versus societal issues in poultry nutrition. Nutr Abstr Rev. 2007; 71:1-5.
15. Liem A, Pesti GM, Edwards Jr HM. The effect of several organic acids on phytat phosphorous hydrolysis in broiler chicks. Poultry Science. 2008; 87:689-693.
16. Mendez-Albores A, Del Rio-García JC, Moreno-Martinez E. Decontamination of aflatoxin duckling feed with aqueous citric acid treatment. Animal Feed Science and Technology. 2007; 135:249-262.
17. Mohamed AA, Habib AB, Eltrefi AME, Shulukh ESA, Abubaker AA, Abdelwahid HH *et al.* Effect of dietary supplementation of increasing levels of organic acid mixture on performance and carcass characteristics of broiler chickens. IOSR Journal of Agriculture and Veterinary Science. 2018; 11(3):54-58.
18. Nian F, Guo YM, Ru YJ, Li FD, Peron A. Effect of exogenous xylanase supplementation on the performance, net energy and gut microflora of broiler chickens fed wheat-based diets. Asian-Australasian Journal of Animal Sciences. 2011; 24(3):400-406.
19. Ramakrishna RR, Platel K, Srinivasan K. *In vitro* influence of spices and spice – active principles on digestive enzymes of rat pancreas and small intestine. Nahrung. 2003; 47:408-412.
20. Ricke SC. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Science. 2003; 82**:**632-639.
21. Rodjan P, Soisuwan K, Thongprajukaew K, Theapparat Y, Khongthong S, Jeenkeawpieam J *et al*. Effect of organic acids or probiotics alone or in combination on growth performance, nutrient digestibility, enzyme activities, intestinal morphology and gut microflora in broiler chickens. Journal of Animal Physiology and Animal Nutrition. 2017; 102(2):931-940.
22. Singh VB. Assessment of performance and gut health of broiler chickens supplemented with phytobiotics, probiotics and organic acids. Ph.D. Thesis, Department of Animal Nutrition, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, (U.P.) India, 2018.
23. Singh VB, Singh VK, Tewari D, Gautam S, Dwivedi D. Growth performance, Haemato-biochemical profile and carcass characterstics of broiler chickens fed a diet supplemented with a natural blend of herbs. Animal Nutrition and Feed Technology. 2016; 16:345-353.
24. Sonu, Sihag ZS, Ahlawat PK, Dalal R. Effect of protease enzyme on the growth performance and carcass traits of broilers fed with DDGS supplemented diet. International journal of current microbiology and applied science. 2018; 7(05):2713-2719.
25. William Horwitz. Official methods of analysis of AOAC International. 17th Ed, Gaithersburg, Md. : AOAC International, Washington DC, USA, 2000.