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EFFICACY OF A PHYTOGENIC FEED ADDITIVE AND AN ANTIBIOTIC GROWTH PROMOTER ON CARCASS AND MEAT TRAITS IN BROILERS

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ABSTRACT

The objective of this twin study was to evaluate the influence of a phytogenic feed additive (PFA) comprising of a selected combination of different plant materials including herbs, spices, essential oils and their extracts and an antibiotic growth promoter (AGP) bacitracin methylene disalicylate (BMD) in comparison to a negative control on carcass and meat traits in broiler chickens.

In Experiment 1, 432 day-old Cobb 400 broilers were randomly assigned to 3 dietary treatments with 12 replications per treatment and 12 birds per replicate over a period of 39 days. In Experiment 2, 120 day-old Cobb 400 broilers were randomly assigned to 3 dietary treatments with 8 replications per treatment and 5 birds per replicate over a period of 39 days. The 3 dietary treatments in both experiments comprised of a control (basal diet only), AGP (basal diet + 225 mg/kg BMD), and PFA (basal diet + PFA Digestarom Poultry 150 mg/kg).Experiment 1: carcass traits were mostly unaffected ($P > 0.05$) by diets except the yield of drumstick which was higher in the AGP and PFA groups as compared with the control ($P = 0.002$). Relative organ weights were also not affected significantly due to supplementation of AGP and PFA to the diet ($P > 0.05$), however, weight of the viscera decreased ($P = 0.004$) in the dietary groups receiving AGP and PFA supplementation. No significant effect of the diets was observed on drip loss and pH of meat, although PFA group tended to have a lower meat pH compared to the other 2 groups ($P > 0.05$).

Experiment 2: supplementation of the PFA significantly increased the moisture content ($P = 0.03$) and Water Holding Capacity (WHC) in the poultry meat compared with the control group ($P = 0.016$). The drip loss (48 h) was significantly less ($P = 0.025$) in the PFA group compared to the AGP group. In conclusion, the evaluated PFA significantly reduced the weight of viscera and increased the moisture content and WHC in meat and consequently reduced the drip loss compared to the BMD group. A reduced FCR and improved weight gain were also registered. The PFA can thus serve as an alternative to the AGP in broiler production.

Keywords: Phytogenic feed additive, antibiotic growth promoter, carcass, meat traits, broilers

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1. INTRODUCTION

There has been a steady increase in the global meat production over the last decades and this trend is projected to continue steadily in the near future due to a steady growth in the human population [1]. Stricter regulations about protection of human health, animal welfare and environmental protection on one side and an increasing demand for animal protein on the other side, make subsequent adaptations necessary for the on-going production process. Due to the rising ban on the use of antibiotic growth promoters (AGP) in food animals, the present trend worldwide to reduce the use ofAGP in poultry rations has put a great pressure on the scientific community as well as the poultry industry to search for viable alternatives. Feed additives of plant origin known as phytogenic feed additives (PFA), comprising of herbs, spices, essential oils (EO), plant extracts and their products have therefore gained considerable interest as alternatives to AGPin food animals. This is attributed to their ability to improve performance by maintaining a healthy gut environment [2]. An alternative to the AGP should ideally have the same beneficial effects as that of an AGP when added to the animal feed. The significance of essential oils present in the PFA in promoting health and enhancing the performance of broilers has been emphasised in a large number of scientific studies. Essential oils containing most of the active substances of the plant have been suggested to improve gut health [3], [4], nutrient digestibility[5] and growth performance[3], [6] in poultry. These various beneficial effects of PFA are attributed to their bioactive molecules like thymol, carvacrol, cineole and capsaicin etc.[7]. Essential oils present in PFA have also been reported to positively influence carcass and meat quality characteristics in food animals [6], [8], [9]. These properties project the PFA as ideal alternatives to the AGP.

In addition to the performance aspects, the objective of these 2 experimental trials was to evaluate the influence of a commercially available PFA comprising of a selected combination of different plant materials including herbs, spices, essential oils and their extracts and an AGPbacitracin methylene disalicylate (BMD) in comparison to a negative control on carcass and meat traits in broiler chickens.

2. MATERIALS AND METHODS

All the animal procedures were conducted in accordance with the prevailing institutional ethical norms of the West Bengal University of Animal and Fishery Sciences, Kolkata, India.

2.1. EXPERIMENT 1

2.1.1. Animals and bird husbandry

A study trial of 39 days duration was conducted with an as-hatched flock of 432 day-old Cobb 400 broilers. The chicks were weighed and randomly assigned to 3 dietary treatments with 12 replications per treatment and 12 birds per replicate (144 birds in each treatment). Birds were raised on litter composed of paddy straw and wood shavings and received maize-soybean meal based diets from 1 to 7 days (starter), 8 to 21 days (grower) and 22 to 39 days (finisher). Feed

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and water was offered ad libitum. Lighting program was 23 h of light for the first 7 d, 20 h until 15 d and 18 h afterwards. The birds were vaccinated against Marek's disease (0 d), Newcastle disease (ND live B1 at 7 d and La Sota at 21 d) and infectious bursal disease (14 d). Temperature was maintained around 32 to33oC during the first 2 weeks and at 27 to 28oC subsequently.

2.1.2. Experimental diets and treatments

Efficacy of the PFA was evaluated on maize-soybean based basal diets (Table 1). The 3 dietary treatments comprised of a control (basal diet only), AGP (basal diet + AGP) and PFA (basal diet + PFA). The AGP used was bacitracin methylene disalicylate (BMD) at the inclusion rate of 225 mg/kg of diet. The phytogenic feed additive used (Digestarom Poultry, 150 mg/kg, Biomin Phytogenics, GmbH, Stadtoldendorf, Germany) comprised of a selected combination of different plant materials including herbs, spices, essential oils and their extracts characterized by a blend of Peppermint (Mentha x piperita), Anise (Pimpinella anisum) and Clove (Syzygium aromaticum). The dietary inclusion level of the PFA was according to the manufacturer's recommendations. No exogenous polysaccharide degrading enzymes and anti-coccidial drugs were added to the diets because these might mask the effects of the PFA, however, a toxin binder was used to prevent mould growth in the stored feed.

Table 1: Experiment 1 - Ingredient composition of basal diet (g/kg, unless stated otherwise).

Item	Prestarter (1 to 7 d)	Starter (8 to 21 d)	Grower (22 to 39 d)
Ingredients			
Ground corn	540.7	567.2	583.4
Soybean meal (460 g CP/kg)	396	362.5	328
Soybean oil	27	33.7	51.1
Calcite powder	12.45	12.45	12.45
Di-calcium phosphate	16.5	16.5	17
DL-methionine	0.55	0.95	1.35
Lysine hydrochloride	0.3	0.2	0.2
Sodium bi carbonate	2	2	2
Salt	2	2	2
Vitamin + mineral premix[1]	2	2	2
Toxin binder	0.5	0.5	0.5

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Calculated composition			
ME, MJ/ kg	11.85	12.14	12.65
Crude protein	223.5	210.4	196.3
Ether extract	52.7	59.8	7.33
Crude fibre	37.5	36.1	3.44
Calcium	9.57	9.47	9.38
Available P	3.18	3.16	3.13
Digestible lysine	11.02	10.19	9.39
Digestible methionine	3.51	3.76	3.99
Digestible methionine + cysteine	6.87	6.73	6.77

1Contained (per kg) retinyl acetate 3.75 mg, 1,25-hydroxy-cholecalciferol 4 mg, DL-α-tochopheryl acetate 30 mg, menadione 4 mg, thiamine propyl disulfide 3 mg, riboflavin tetrabutyrate 8 mg, riboflavin tetrabutyrate 8 mg, methylcobalamin 0.025 mg, sodium pantothenate 15 mg, pyridoxine 5 mg, niacin 60 mg, biotin 0.2 mg, folic acid 2 mg, manganese 40 mg, iron 30 mg, zinc 25 mg, copper 3.5 mg, iodine 0.3 mg, selenium 0.15 mg, choline chloride 200 mg.

2.1.3. Measurement of Production traits, meat traits and carcass parameters

Birds were individually weighed at 7, 21 and 39 d which designated the prestarter, starter and grower periods respectively. Body weight gain (BWG), total feed intake (FI), average daily feed intake (ADFI) and feed conversion (FI: BWG) were calculated pen wise for each period and for the overall period. Mortality was recorded daily and the data was adjusted accordingly.

At 39 d, 12 birds having BW closest to the mean weight of the group were selected from each dietary treatment. The birds were weighed and killed by cervical dislocation, followed by exsanguination. After the removal of feathers, viscera, shanks and neck, the weights of the eviscerated hot carcass, breast, drumsticks, thighs and organs (viscera, liver, gizzard and heart) were measured. Carcass yield and yields of breast, thighs and drumsticks relative to live weight (g/kg) were measured. Breast fillets (approximately 200 g) were stored at 4oC for 24 h for determination of pH, drip loss, moisture and crude protein contents. Drip loss and pH were determined according to [10]. Pieces of breast fillet (50 g) were stored at 4oC for 48 h and the loss of weight as the percentage of the original sample weight was determined. This loss was equivalent to the drip lost from the stored meat. Meat pH was measured at 6 different locations across the sample surface with a pH meter (AB15, Thermo Fisher scientific, Waltham, MA, USA). The average represented the ultimate pH of the sample.

2.2. EXPERIMENT 2 (challenge with enteric pathogens)

2.2.1. Animals and bird husbandry

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A second 39 day experimental feeding trial was conducted with an as-hatched flock of 120 day-old male Cobb 400 broiler chicks in accordance with the prevailing institutional ethical norms at the West Bengal University of Animal and Fishery Sciences, Kolkata, India. The chicks were weighed individually and assigned randomly to 3 dietary treatments, each consisting of 8 replicate cages with 5 birds per replicate. Immediately after arrival, swabs from the cloacae were collected from all chicks and analysed for Salmonella and E. coli counts. The chicks were found negative for Salmonella but E. coli was detected in 2 chicks, which were excluded from the experiment. All other bird housing and husbandry conditions and practices were similar to that of experiment 1 as described under the sub-heading number 2.1.1. above.

2.2.2. Experimental diets and treatments

Efficacy of the PFA was evaluated on maize-soybean based basal diets (Table 2). The 3 dietary treatments evaluated were also exactly the same as in the Experiment 1 described in detail under the sub-heading 2.1.2. Experimental diets and treatments.

Table 2: Experiment 2 - Ingredient composition of basal diet(g/kg, unless stated otherwise).

Item	Pre-starter (1 to 7 d)	Starter (8 to 21 d)	Grower (22 to 39 d)
Ingredients			
Ground corn	605.2	632.9	701
Soybean meal (460 g CP/kg)	349.8	316.8	248.6
Soybean oil	9.7	19.8	22.7
Calcite powder	9	8.4	8
Di-calcium phosphate	12.9	9.9	7.9
DL-methionine	2.5	2.3	2
Lysine hydrochloride	2.2	1.9	1.9
L-threonine	0.8	0.5	0.9
Sodium bi carbonate	1.5	1.5	1.5
Salt	2.8	2.4	1.9
Choline chloride 600 g/kg	0.8	0.8	0.8
Toxin binder[1]	1	1	1
Mineral premix[2]	1	1	1

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Vitamin premix[3]	0.5	0.5	0.5
Antioxidant	0.1	0.1	0.1
Phytase 500 ftu/kg feed)[4]	0.1	0.1	0.1
NSPase enzyme[5]	0.1	0.1	0.1
Nutritive values (calculated unless stated otherwise)			
ME, MJ/ kg	12.1	12.55	12.97
Crude protein[6]	210.4	196.7	172.2
Ether extract[6]	36.1	46.9	51.5
Crude fiber[6]	37.2	35.8	33.1
Calcium	9	8	7.2
Available P	4.5	4	3.6
Dry matter[6]	942.6	942.1	942.2
Total ash[6]	68.9	69.1	67.8
Standardized ileal digestible amino acids			
Lysine	12	11	9.5
Methionine	4.4	4.18	3.8
Methionine + Cysteine	8.4	8.03	7.22
Threonine	7.7	7.04	6.46
Tryptophan	1.9	1.9	1.7
Arginine	12.6	11.8	10.45
Isoleucine	7.8	7.37	6.55
Valine	9	8.5	7.5

1 Containing diatomaceous earth and yeast cell wall (mannan oligosaccharides).2 Containing manganese 40 mg, iron 30 mg, zinc 25 mg, copper 3.5 mg (all as sulfate salts), iodine 0.3 mg (as potassium iodide), selenium 0.15 mg (as sodium selenite).3 Contained (per kg) retinyl acetate 3.75 mg, 1,25-hydroxy-cholecalciferol 4 mg, DL-α-tochopheryl acetate 30 mg, menadione 4 mg, thiamine propyl disulfide 3 mg, riboflavin tetrabutyrate 8 mg, riboflavin tetrabutyrate 8 mg, methylcobalamin 0.025 mg, sodium pantothenate 15 mg, pyridoxine 5 mg, niacin 60 mg, biotin 0.2 mg, folic acid 2 mg.4Escherichia coli phytase with minimum activity of 5000 ftu/g., 5 Containing endo 1, 4-β-xylanase and endo 1, 4-β- glucanase activity.6 Estimated values.

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2.2.3. Pathogen challenge

In order to evaluate the effect of the dietary treatments especially during periods of infectious stresses under field conditions, the birds were pushed to more of a diseased state by inoculating with enteric pathogens. At 28 d all birds were challenged with 2.5 x 1010 colony forming units (cfu) of S. enteritidis 0363P (ATCC 14028) and E. coli (ATCC 25922) through oral inoculation [11].

2.2.4. Measurement of production traits, meat traits and carcass parameters

The chicks were individually weighed for their empty body weight following a 16h fast at weekly intervals and live weight (LW) and live weight gain (LWG) were calculated cage wise. Cage average for feed intake (FI) was determined every week and feed conversion ratio (FCR) was calculated for each cage as the ratio between feed intake and LWG. All birds were killed on 39 d by cervical dislocation and the feathers, viscera, shanks and neck were removed. The weights of the eviscerated hot carcass, breast, drumsticks and thighs were expressed relative to live weight. The whole breast was stored as fillet at 4oC for 24 h for determination of pH, drip loss, water holding capacity (WHC), moisture and protein content. Meat pH was measured as per [10] at 6 different locations across the sample surface with a pH meter (AB15, Thermo Fisher scientific, Waltham, MA, USA). The average represented the ultimate pH of the sample. Moisture was determined by drying approximately 100 g minced breast meat sample at 98oC for 48 h. The dried meat was cooled to room temperature in a desiccator prior to taking final weights. Meat protein (Nitrogen x 6.25) was determined by Kjeldahl distillation method taking 1 g of minced meat [12]. Drip loss was determined as per[10] by keeping breast fillets (50 g) at 4oC for 48 h and expressing the loss of weight as percentage of the original sample weight. For WHC, meat samples (200 and 400 mg) were placed on 11 cm diameter filter paper between plexi glass plates and pressed at 2,000 psi for 1 min [10]. The outline area of the expressible juice and the meat film was traced, and the 2 areas were determined. Higher expressible juice percentage is related to lower WHC. Expressible juice, as a percentage, was calculated as follows:

Expressible juice (%) =100 ×[(Total juice area- Meat film area) ×Water/Inch2 filter paper]/Moisture of sample (mg).

2.3. Calculation and statistical analysis

The cages were the experimental units and all data were pooled cage wise unless specified otherwise and expressed as mean and pooled standard error of means (SEM). The data were subjected to one way analysis of variance (SPSS version 10.1) with the diets as the factor and when found significant the means were separated by Tukey´s test. Significant differences were accepted if P □ 0.05.

3. RESULTS

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The birds were healthy during the experimental trials. Despite enteric bacterial challenge in the second experiment, no mortality occurred during the trial.

3.1. Results experiment 1

Carcass traits were mostly unaffected (P> 0.05) by diets (Table 3) except the yield of drumstick which was higher in the AGP and PFA groups as compared with the control (P = 0.002). Relative organ weights were also not affected significantly due to supplementation of AGP and PFA to the diet (P> 0.05), however, weight of the viscera decreased (P = 0.004) in the dietary groups receiving AGP and PFA supplementation. Weight of the heart tended to be increased in the AGP and PFA groups as compared with the control group (P =0.094). No significant effect of the diets was observed on drip loss and pH of meat although PFA group tended to have a lower meat pH compared to the other 2 groups (P> 0.05).

Table 3: Experiment 1 - Carcass traits and relative organ weight (g/g live weight) of experimental birds supplemented with either an antibiotic or a phytogenic growth promoter

Components	Dietary treatments[2]			SEM	*P*-value
	Control	AGP	PFA		
Yield of carcass cuts, g/kg live weight					
Carcass yield	714.7	719.8	728.1	0.49	0.566
Breast	180.3	183.5	187.7	0.25	0.188
Frame	246.3	228.8	238.2	0.32	0.244
Thigh	72.6	77.3	74.7	0.13	0.35
Drumstick	136.7 [a]	149.9 [b]	153.9 [b]	0.16	0.002
Relative organ weight, g/kg live weight					
Viscera	113.3 [b]	82.1 [a]	80.8 [a]	0.14	0.004
Liver	23.9	22.8	22.3	2.21	0.584
Gizzard	48.4	49.5	43.5	4.31	0.253
Heart	6.48	7.88	7.71	0.068	0.094
Drip loss (24 h), %[3]	2.11	2.12	2.06	0.13	0.521
Meat pH (24 h)[3]	6.01	5.89	5.81	0.044	0.298

AGP = bacitracin methylene disalicylate; PFA = Digestarom Poultry, a,bMeans with dissimilar letters in a row varied significantly (P< 0.05). [1]Means of 12 birds per treatment. Birds were selected randomly and killed

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at 39 d of age.[2]The 3 dietary treatments comprised of a control (basal diet only), AGP (basal diet + AGP) and PFA (basal diet + PFA). [3] Measured after 24 of storage at 4oC.

3.2. RESULTS EXPERIMENT 2

Supplementation of the PFA significantly increased the moisture content (P=0.03) and WHC (P=0.016) in the poultry meat compared with the control group. Consequently the drip loss (48 h) was significantly less (P=0.025) in the PFA group compared to the AGP group (Table 4). Other carcass traits remained largely unaffected by the dietary treatments (P> 0.05).

Table 4: Experiment 2 - Carcass and meat traits in broilers supplemented with an AGP or a PFA and infected with Salmonellaand Escherichia coli (means represent 8 birds from each dietary treatment).

Parameter	Dietary treatments[1]			SEM	*P*- value
	Control	AGP	PFA		
Relative yields, g/kg live weight					
Carcass yield	741.7	751.1	761.7	6.18	0.448
Breast	187.4	187.3	189.5	3.91	0.968
Drumsticks	90.9	97.2	97.4	1.9	0.311
Thigh	76.3	80.4	84.7	2.11	0.289
Organ weights, g/kg live weight					
Spleen	1.77	1.56	1.68	0.07	0.48
Heart	4.21	4.26	4.35	0.12	0.897
Liver	21.36	20.61	22.78	0.52	0.215
Bursa	2.11	2.54	2.22	0.01	0.375
Gizzard	44.73	45.7	40.36	1.52	0.316
Viscera	75.62	74.81	72.39	2.19	0.836
Meat traits					
Moisture, g/kg	728.6[a]	751.3[ab]	763.8[b]	5.59	0.03
Protein, g/kg	184.3	184.5	187.1	1.11	0.526
PH[2]	6.27	6.35	6.3	0.02	0.457
Water holding capacity, %[2]	38.6 [a]	42.5 [ab]	44.8 [b]	0.92	0.016

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Drip loss, %[2]	2.35[b]	2.22[b]	1.73[a]	0.1	0.025

AGP = bacitracin methylene disalicylate; PFA = Digestarom Poultry [a,b]Means with dissimilar letters in a row varied significantly (P< 0.05).n1The 3 dietary treatments comprised of a control (basal diet only), AGP (basal diet + AGP) and PFA (basal diet + PFA). 2 Measured after 24 of storage at 4oC.

Table 5: Experiment 1 - Performance of broilers supplemented with a PFA or an AGP from 1 to 39 d

Components	Dietary treatments[1]			SEM	*P*-value
	Control	AGP	PFA		
Body weight gain, g	1,896.9 [a]	1,969.5 [b]	2,018.2 [b]	14.41	0.001
Feed intake, g	3,789.5	3,800.2	3,751.5	9.81	0.101
Feed conversion ratio	2.002 [b]	1.931 [ab]	1.860 [a]	0.015	0.0001

AGP = bacitracin methylene disalicylate; PFA = Digestarom Poultry [a,b]Means with dissimilar letters in a row varied significantly (P< 0.05). ,[1]The 3 dietary treatments comprised of a control (basal diet only), AGP (basal diet + AGP) and PFA (basal diet + PFA).

Table 6: Experiment 2– Body weight gain and feed conversion ratio of broilers supplemented with a PFA or an AGP from 1 to 39 d

Components	Dietary treatments[1]			SEM	*P*-value
	Control	AGP	PFA		
Body weight gain, g	1,971.5 [a]	2,028.9 [ab]	2,096.9 [b]	23.11	0.05
Feed conversion ratio	1.816 [b]	1.729 [ab]	1.632 [a]	0.026	0.006

AGP = bacitracin methylene disalicylate; PFA = Digestarom Poultry ,[a,b]Means with dissimilar letters in a row varied significantly (P< 0.05). [1]The 3 dietary treatments comprised of a control (basal diet only), AGP (basal diet + AGP) and PFA (basal diet + PFA).

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4. DISCUSSION

Phytogenic feed additives have attracted increasing interest as an alternative feeding strategy to replace the AGP particularly in the European Union, where use of AGP as feed additives is completely banned since 2006 because of a suspected risk of generating microbiota with increased resistance to the antibiotics used for therapy in humans and animals [2]. PFA have also been reported to positively influence carcass and meat quality characteristics in food animals [6], [13]. Results of the present investigation revealed that the yield of drumstick was higher in the AGP and PFA groups as compared with the control. Although the relative organ weights were not affected significantly due to supplementation of AGP and PFA to the diet, however, weight of the viscera decreased in the dietary groups receiving AGP and PFA supplementation. The reduction in viscera weight by dietary supplementation of AGP and PFA implied a reduction in the energy required to maintain the gut, thereby leaving more energy available for productive processes such as body weight gain and feed conversion ratio. These findings are substantiated in the present studies (Table 5). These findings are further supported by the fact that despite an enteric pathogen challenge in the second experimental study, supplementation with the PFA improved body weight and FCR of broilers (Table 6). Plant extracts and essential oils reportedly improved broiler performance[5], [7],[14].

Supplementation of the PFA significantly increased the moisture content and WHC in the poultry meat compared with the control group. Consequently the drip loss (48 h) was significantly less in the PFA group compared to the AGP group. The ability of meat to retain inherent water known as WHC is an important property of fresh meat as it affects both the yield and the succulence of the end product. This characteristic can be described in several ways, but in fresh products that have not been extensively processed, it is often described as drip loss or purge. The mechanism by which drip or purge is lost from meat is influenced by both the pH of the tissue and by the amount of space in the muscle cell and particularly the myofibril that retains the water. Numerous factors affect both the rate and the amount of drip. Of extreme importance is the metabolic state of the live bird at the time of harvest, which is influenced by the genetic make-up of the bird, characteristics of the muscle and the production regime and standards [15].
The aqueous solution that is lost from post-mortem muscle (drip) contains significant amount of proteins, myoglobin, glycolytic enzymes, other sarcoplasmic proteins, amino acids and water-soluble vitamins [15]. Hence, it can be well understood as to how important is the reduction of drip loss as well as WHC of meat from the nutritional as well as the eating quality aspect of meat for the consumers.

CONCLUSION

The overall results of these two studies reveal that the PFA evaluated when added to a maize-soybean meal based coccidiostat free broiler diet:

i. Is equally effective like the AGP used, as regards the carcass and meat traits, coupled with performance of the birds are concerned and thus can be considered in dietary regimens of poultry in place of the antibiotic growth promoters.

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ii. Significantly increased the moisture content and WHC in meat and consequently reduced the drip loss in 48 h compared to the BMD group. This advantageous effect of the PFA is not only of nutritional importance but also more of considerable economic importance, benefit for slaughter houses, producers, meat purchasers and consumers of poultry meat.

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