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Effects of Antibiotic Growth Promoter Free Production on The performance, Blood Properties, and Humoral Immune Response of Broiler Chicken

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Abstract

THE present study assessed the effect of replacing antibiotic growth promoters (AGP) with an AGP-free program on broiler chicken performance and economic returns. Therefore, these studies were conducted in two separate experiments. The first experiment was started at the BLRI research farm to determine the use of antibiotics in the broiler chicken's feed and water. 600-day-old chicks (Lohmann) were casually distributed to 3 treatments, with 10 replicates of 20 chicks. Broiler's usual diet throughout the rearing period was as follows: control (received no AGP), antibiotics (received 0.1% antibiotics as growth promoters), and AGP-free (received multivitamins, acidifiers, calcium, zinc, liver tonic, and saline). In the second experiment, a training program was arranged for the farmers on safe broiler production before the onset of the experiment. The farmers were then categorized into Group 1 (farmers produced their broilers following existing practices and using antibiotics as growth promoters) and Group 2 (farmers reared their broilers using the AGP-free program). The results demonstrated that the antibiotic and AGP-free group showed higher body weight and weight gain than the control group. A significant difference was investigated in the feed intake of the three treatment clusters. Based on the performance of broiler chicken production, the application of the AGP-free program saves production costs 7.50 Tk/birds than that of the conventional system using antibiotics. The findings show that it could be possible to commercially raise broiler chickens using an AGP-free package in Bangladesh.

Keywords: AGP-free programs, Antibiotic, Performance, Safe broiler.

Introduction

The poultry industry has gained momentum through increased investments in rural and commercial sectors, amounting to Tk 50,000 crore, and has formed hire occasions for around 8 million people [1]. In addition, by providing accessible and cheap protein sources of diets for the growing and marginal population, this sector promotes the achievement of the sustainable development goals [2]; especially goal number 2 is targeting to end hunger, achieve food safety and advance nutrition, and progress maintainable agriculture. Modulatory factors such as population growth, higher income status, and urbanization are stimulated for faster development and will contribute to the sector in the future. Hence, more capital, productivity, and profitability make the sector attractive to domestic entrepreneurs and foreign investors. Backyard farming has evolved into a commercial industry with modern equipment, imported breeds, and structured marketing [3].

After sustained efforts and policy changes, the poultry industry has achieved self-sufficiency in meat production. According to Larive and Light Castle [4], broiler chicken is the fastest-growing and most-consumed poultry, comprising more than 58.39 percent of the total chickens in Bangladesh. The growth in this segment is directly partial to the recent expansions in the current food dispensation business,

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particularly the emergence of fast-food chains operating online, the introduction of trendy food items into people's eating habits, and subsequently, the demand for large numbers of chickens, resulting in the establishment of over 53,000 broiler farms presently [5]. The petition for poultry meat in Bangladesh has grown meaningfully, showing an increase in per capita poultry meat consumption by 8.5kg per year, sharing about 28% and 54% of total meat consumption globally and in the country, respectively. In contrast, broiler meat consumption increased by 6.3kg annually [6].

To keep the production and consumption of broiler chicken on track and to meet the rising demand in the country, the poultry industry has traditionally relied on antibiotic growth promoters (AGPs) to develop growth performance and maintain health in broiler chickens [7]. However, misuse of AGPs in the broiler sector is a significant concern due to their potential impacts on human health, animal welfare, and the environment [8]. Due to the widespread use of antimicrobials, antimicrobial resistance (AMR) to pathogens poses a substantial concern to human fitness due to its zoonotic significance, frequently resulting in considerable economic loss for the meat industry [9]. Besides the AMR effect, AGPs can disturb the natural equilibrium of gut microbiota in broilers, leading to reduced microbial diversity, which can negatively impact nutrient absorption and overall gut health, potentially causing dysbiosis [10]. Furthermore, AGPs can have an antibiotic residue effect on meat, which can harm the consumers taking the treated broilers' meat by causing allergic reactions and developing the AMR genes in the environment [11]. In Bangladesh, the use of AGPs in broiler chicken farming is a significant concern due to its widespread use, AMR contribution, residual effects on meat, and environmental hazards, focusing on a time-demanding choice of alternatives. Additionally, there is a lack of stringent regulation and enforcement regarding the use of AGPs in this sector, causing the issue to be more profound to control [12]. The demand for substitute methods to replace AGPs without compromising broiler chicken productivity and health is increasing. In poultry production, numerous alternatives to AGPs can aid in maintaining health and enhancing growth performance. Probiotics, prebiotics, phytobiotics feed additives, organic acids, enzymes, essential oils, and immunomodulators are among the most utilized substitutes [13]. These alternatives can be applied individually or in a combination of approaches to obtain poultry performance and fitness [14]. The choice of this substitute varied on factors such as the target requirement of the flock, cost, and availability.

In previous research, several approaches were prescribed for broiler chickens alternative to AGP-free programs using prebiotics [15], probiotics [16], phytobiotics [17], and plant-based additives, considering the reduction of AGP effect on broiler meat to enhance growth without the negative impact associated with antibiotics. The novelty of this research paper stands out for its comprehensive assessment of an AGP-free program for broiler production, focusing on a holistic approach, incorporating health, performance, economic, and training components, all within a practical and locally relevant framework, providing actionable insight that could be the transition to AGP-free poultry production in Bangladesh and similar regions. Therefore, the objectives of this study mainly focused on using this AGP-free program by using the below-mentioned additives packages on the growth performance, blood properties, and humoral immune response of broiler chicken in Bangladesh.

Material and Methods

Experiment 1

The study was carried out at the BLRI investigation farmhouse to determine the safest alternative to antibiotic use in broiler feed and water. A total of 600-day-old Lohmann chicks were randomly distributed to three treatment groups in a complete randomized design (CRD) with 10 replicates per treatment and 20 chicks per replicate.

The three treatment groups included, the first group (control) was given no antibiotics, the second group which was given antibiotics at 0.1% rate as growth promoters and the third group which received multivitamins, acidifiers, calcium, zinc, liver tonic, and saline at different ages without antibiotics. Standard management practices were applied during the experimental period.

An ideal brooding temperature of 32-33^{0}C was maintained for the first week and reduced to 3^{0}C per week. Stocking density of 1.0 sq. ft./bird for the winter season and 1.2 sq. ft./bird for the summer season up to 5 weeks of age. The composition of the experimental diet provided at various stages of birds is shown in Table 1. The third treatment group for AGP-free broiler production followed the schedule in Table 2. Fresh and dry rice husk with a thickness of 2 was used as litter materials to ensure bird comfort. Strict biosecurity measures were implemented around the experimental unit and birds were immunized against common diseases using the vaccines across all groups. Uniform vaccine, diet, and management practices were applied to each group. The relative humidity was kept at 70%, and continuous lighting was provided throughout the study. At the end of the experiment, 1 ml of blood was collected from the wing veins of 10 birds per group using a syringe with a Hamilton 22-gauge needle (0.70 mm diameter). The blood was obtained to stand at room temperature (RT) for 30 minutes, after which the serum was separated by centrifuging at 2,200 × g for 15 min at 4 ^{0}C. After the serum separation process, it was preserved at -80 ^{0}C until immunoglobulin analysis. The blood lipid profiles and liver enzyme activities were determined utilizing suitable protocols, and immunoglobulin levels were evaluated with a commercial kit using the double antibody sandwich ELISA method. For meat yield parameters, ten birds from each treatment group, with body weights close to the group average were selected. The birds were humanely slaughtered and bled for 2 minutes, semi-scalding (immersed in water at 51-55°C for 120 seconds), and hand-pinned to remove feathers. Subsequently, the head, viscera, shank, and giblets (heart, liver, gizzard, and abdominal fat) were detached to determine meat yield. The dressed birds were segmented into breasts, thighs, drumsticks, and wings each part individually weighed and recorded for all treatment groups. The breast muscle was utilized to measure meat color by employing a Minolta reflectance colorimeter (Minolta Chroma Meter CR-300, Japan). Before the color measurement, the breast muscle was sliced and air-dried for 15 minutes (RT). The meat color was measured three times using a white ceramic tile as the standard, yielding the following results: L (lightness) = 92.30, a (redness) = 0.32, and b (yellowness) = 0.33. The meat color was expressed in terms of Hunter values. To determine pH, 5 g of the meat sample was taken in a filter bag, and 15 ml of buffered peptone water was mixed with the sample and homogenized the samples for two minutes before measuring the pH of the solution through a benchtop pH meter. To estimate the cooking loss (CL%) percentages, five meat samples (n = 5) weighing an average of 5.00 g were obtained from each treatment group, vacuum packed, and stored at 4 ± 1 °C. The frozen meat samples were taken out of the freezer and gently wrapped in paper towels, and their weight (Wi) was measured. Next, the meat was cooked in a water bath preheated to 80°C for 30 min. Once the internal temperature of the samples got 78°C, as indicated by a probe thermometer (HI 145-00 thermometer, HANNA instruments), they were left to cook for an additional 10 min to confirm that all the samples had reached a uniform final internal temperature. Subsequently, the meat samples were removed from the water bath, allowed to cool to RT, removed from the plastic bag, carefully dried with paper towels without applying pressure, and then re-weighed (W_f) after 24 hours. The CL % was measured using the procedures in the following equation.

$$CL\ \% = \left[\frac{W_i - W_f}{W_i}\right] x100$$

Where Wi represents the initial weight of the meat sample before being cooked in the water bath (g), and W_f represents the weight of the meat sample after being cooked in the water bath (g).

Experiment 2

The study was conducted at farmers' household levels, and farmers were involved in performing this experiment after site selection. A training program regarding safe broiler production was also provided. After that, the farmers were separated into two groups. In group 1, the farmers traditionally produced their broilers using antibiotics, while in group 2, farmers produced their broilers using an AGP-free program. The same managemental procedure was followed as in experiment 1.

Data analysis

The data analysis used the General Linear Model (GLM) procedure with SAS software. This procedure was applied in the study using a completely randomized design (CRD), and any variations in the data were identified using Duncan's multiple range test (DMRT) [18]. In the analysis of growth performance parameters, pens were used as replicates, while randomly selected birds were the replicates for carcass and blood parameter analysis. A p-value of less than 0.05 was considered significant, and means were compared using the DMRT procedure.

Results

The effect of the AGP-free program on the body weight, weight gain, feed intake, and FCR of broilers is presented in Table 5. A significant difference ($P < 0.05$) was observed in body weight at 21 and 35 days. However, no significant difference in body weight was observed in the first two weeks. Antibiotics supplemented with 0.1% as a growth promoter and AGP-free group showed higher body weights, 1072.43 g, and 1056.89 g at 21 days and 2349.20 g and 2319.23 g at 35 days, respectively. However, the control group showed a lower body weight of 1001.55 g and 2260.07 g at 21 and 35 days, respectively. While considering the weight gain of the broiler, a significant difference ($p < 0.01$) has been observed in the 2nd week on-ward. A similar trend was found in overall weight gain. Antibiotics supplemented with 0.1% as growth promoter group have higher body weight gain (2299.55 g) followed by AGP-free group (2269.73 g) and control group (2210.42 g) respectively. The effect of the AGP-free program on the FCR of birds reared for 8 to 35 days was found to have a significant impression ($P < 0.05$). In the first week, no significant difference was stated ($P > 0.05$), or the overall FCR.

The effect of the AGP-free diet on the different blood properties of red blood cells, lipid profile, liver enzyme activity, and humoral immune response in broiler chicken is shown in Table 6. Statistically, the ESR count (mm in 1st h), HDL mg/dl), IgA (µ/ml) and IgG (µ/ml) were significantly varied ($P <0.05$) among the treatment groups. However, no significant difference ($P <0.05$) was observed in other blood properties of broiler birds supplemented with AGP-free and antibiotic diets. The higher HDL content was found in the free group (118.78 mg/dl) and control group (118.08 mg/dl), and the lowest HDL content was found in the antibiotic group (97.68 mg/dl). No significant difference was found in other lipid properties parameters, i.e., glucose, cholesterol, LDL, and total protein content in the three treatment groups. Significant differences have been observed in humoral immune response parameters, i.e., IgA and IgG, among the three treatment groups. The highest IgA was found in the AGP-free group (21.42 μ/ml) than that of the control group (20.87 μ/ml), while the lowest IgA was found in the broilers supplemented with the antibiotics (19.72 μ/ml).

The AGP-free program's effect on broiler meat's carcass characteristics is presented in Table 7. There were significant differences in live body weight in the three treatment groups at the time of slaughter. The higher body weight was found in the antibiotic group (2349.20 g) and AGP-free group (2319.23g), and the lowest body weight was found in the control group (2260.07 g). However, no significant difference was observed in other carcass parameters of carcass weight (g), dressing percentage, breast meat (% BW), thigh meat (% BW), drumstick (% BW), wing (% BW), liver (% BW), heart (% BW), fat pad (% BW), gizzard (% BW), spleen (% BW), shank (% BW), and bursa (% BW). Numerically, the dressing percentage was in the range of 70 to 72 % of broiler meat obtained in the three treatment groups.

Consumers consider meat color as a primary factor in determining meat quality. Therefore, this experiment also investigated the meat quality of broiler chicken supplemented the feed with antibiotics and an AGP-free diet along with control. The AGP-free program's effect on broiler chicks' meat quality is explained in Table 8. Non-significant difference was detected in cooking loss among the three treatment groups. A significant difference ($P < 0.01$) in meat redness (a) was observed in the three treatment groups, while the higher redness of meat was observed in the antibiotic group (2.68), followed by the AGP-free group (1.99) and the control group (1.64). Significant differences have been observed in the pH level of broiler chicken treated with antibiotics and the AGP-free program in the three groups. Because the pre-slaughtering and slaughtering process of the animal was not controlled in this investigation, statistically significant variations in pH values were anticipated among the treatments. However, the antibiotic and AGP-free groups tended to have a lower cooking loss ($P = 0.421$) and greater yellowness ($P = 0.565$) compared to control. It was assumed that there is a connection between the growth-enhancing effect of antibiotics given at a sub-therapeutic dose, the diameter of muscle fibers, and the tenderness of the resulting meat. It is logical to anticipate that this meat will have a higher cooking loss compared to the meat from untreated birds. However, there were no significant differences in the meat color in terms of lightness (L), yellowness (b), and cooking loss (Table 8, $P < 0.05$). However, this study did not find a compelling reason for the cooking loss of the meat from the birds treated without AGP to be insignificantly ($P > 0.05$) lower (10.30%) than that of the meat from the control group (12.01%).

Experiment 2

The results of body weight, feed intake, weight gain, FCR, and livability under the AGP-free and conventional programs are presented in Table 9. No significant difference was observed in body weight, weight gain, feed intake, FCR, and livability. The results of the present study indicated that the growth performance in both programs was similar due to AGP-free programs containing some components that may enhance the growth performance, the same as antibiotics.

The economic returns of the AGP-free program and conventional practices are presented in Table 10. The fixed costs such as depreciation on sheds, feeders, drinkers, and other essential equipment, were almost identical in both programs, with the AGP-free system costing 6094.77 Tk and the conventional system 6207.68 Tk. However, variable costs were notably lower in the AGP-free program, mainly due to reduced medication expenses, which amounted to 6405 Tk compared to 17260 Tk in the conventional program. This reduction in medication costs resulted in a lower total variable cost of 197694.36 Tk for the AGP-free program, in contrast to 208726.43 Tk for the conventional program. While both programs had similar gross returns 252580.02 Tk for the AGP-free program and 256373.05 Tk for the conventional program the AGP-free system incurred lower total costs of 203789.14 Tk, compared to 214934.11 Tk for the conventional system. This cost advantage allowed the AGP-free system to generate higher net returns (48790.8 Tk) and a greater return over variable costs (54885.66 Tk), while the conventional program had net returns of 41438.94 Tk and return over variable costs of 47647.94 Tk. The input-output ratio for the AGP-free program was more favorable at 1.36, compared to 1.30 for the conventional program, suggesting better cost efficiency. Both systems exhibited similar fixed cost shares in the input-output ratio (0.04), but the AGP-free program demonstrated slightly higher variable cost efficiency was noted at 1.32 vs. 1.26 for the conventional program.

Discussion

This study showed that dietary supplementation significantly impacts broiler body weight and weight gain, with both Antibiotics supplemented (0.1%) and AGP-free groups exhibiting higher body weights at both 21 and 35 days compared to the control group. This may be due to antibiotics as growth promoters and the AGP-free group's antibacterial and growth-enhancing capabilities [19]. The higher body weight gains observed in both the antibiotic-supplemented and AGP-free groups highlight their potential to enhance growth rates through improved feed efficiency and metabolism. In this experiment, body weight, weight gain, and feed intake were found to be similar with antibiotic and AGP-free programs compared to control, consistent with the results obtained [19]. The results obtained from previous studies suggest that both treatments improved growth and immune status offering benefits similar to sub-therapeutic antibiotics used in broiler production [20-21].

Notably, the antibiotic group achieved the highest weight gain (2299.55 g), with the AGP-free group (2269.73 g), closely following (2269.73 g), highlighting the potential of AGP-free additives as a viable alternative. These results supported growing evidence that non-antibiotic additives can support growth and health in poultry, addressing concerns over antibiotic resistance [22]. The researchers explained that birds fed probiotics showed significant growth performance (P< 0.05) compared to those fed antibiotic growth promoters and traditional diets [23]. The result also demonstrated that ginger and its powders increased body weight and feed conversion, while organic acids in feed enhanced the growth, feed conversion rate, and consumption of broiler chicken [25]. Adding organic acids to drinking water protects young chicks against *Campylobacter* infection [26]. Additionally, essential oils can replace growth-promoting antibiotics like avilamycin in improving chicken performance [27], meat quality, carcass quality, and overall health, while vitamin-mineral premix supplementation enhances weight gain and feed efficiency in broilers [28]. An experiment reported that adding 0.625% Calcium to the diet increased body weight and optimized the feed conversion ratio [29]. The AGP-free program significant impression on the FCR of birds from 8 to 35 days (P < 0.05), with no significant difference in the first week in overall FCR. Feeding birds antibiotics during that time significantly reduced the FCR [19]. The findings were similar to those of [30] who studied the effect of ginger root powder growth performance, and serum constituents in broiler chickens. The researchers noted no significant variance in feed consumption between the control group and experimental groups. The previous study [20] reported increased feed intake in broilers fed probiotics and ginger powder, a finding also corroborated [31], who found higher feed consumption in broilers given ginger

The research found that the FCR was largely unaffected by AGP-free feeds and antibiotics compared to the control group. This finding was consistent with [32], which observed that supplementing broiler diets with zinc sulfate increased body weight gain without affecting mortality or feed efficiency, as well as [33] which reported similar improvement with zinc oxide and sodium selenite supplementation. Supplementation with essential oils has also been demonstrated to enhance feed efficiency by lowering FCR, as noted by [34], and ginger supplementation also positively affects FCR in broilers [35]. However, in contrast to the present results [22] found that broilers fed probiotics had significantly better FCR (P< 0.05) compared to AGP or traditional diets.

The elevated HDL levels in the AGP-free and control groups, compared to the lower levels in the antibiotic group, suggest AGP-free diets might promote beneficial lipid profiles in broilers. The findings were similar to those of [36], who found increased hemoglobin, RBC count, and hematocrit percentage, unlike [22] which assessed probiotics' effect on chickens' hematology. Saeid et al. [37], found that ginger extract significantly reduced cholesterol levels in broiler blood. The ESR in the antibiotic group suggests an inflammatory response, while the increased IgA and IgG levels in the AGP-free group highlight that AGP-free diets might support immune health in broilers. Elevated levels of IgA and IgG indicated enhanced mucosal and systemic immunity, helping broilers better combat pathogens. This supports findings that natural growth promoters in AGP-free diets can boost immune function and overall poultry without negative effects [38].

A study was conducted by Debnath et al. [38] and found no significant difference was observed in ALT and AST levels while evaluating the impact of polyherbal liver tonic-Xlivpro premix on broilers' growth and blood properties. This research fed the day-old chicks (n=60) by dividing them randomly as a normal basal diet with no liver tonic premix and a basal diet supplemented with herbal liver tonic Xlivpro premix. The AGP-free diet led to the highest IgA levels (21.42 μ/ml), suggesting a strengthened mucosal immune response. The presence of natural compounds in AGP-free diets supports gut health and stimulates IgA production by promoting a balanced microbiome and a favorable immune environment [39]. The antibiotic-supplemented group displayed the highest IgG levels (2.25 μ/ml), suggesting a robust systemic immune response, possibly the changes in gut microbiota. Antibiotics can enhance growth by modulating gut flora while increasing humoral immunity by gastrointestinal infection suppression [40]. The AGP-free group showed intermediate IgG levels (2.18 μ/ml) indicating a balanced manner for systemic immunity without the immune activation seed with antibiotics. The combination of high IgA and moderate IgG levels suggests a comprehensive immune response which could contribute to overall health and disease resistance in broilers.

The higher live body weights in both the antibiotic (2349.20 g) and AGP-free (2319.23 g) groups compared to the control group (2260.07 g) suggest that both dietary interventions effectively promoted growth. This results with prior studies demonstrating that AGP-free diets, often fortified with natural growth-promoting compounds, can achieve growth outcomes comparable to those seen with antibiotics [41]. However, the lack of significant differences in carcass parameters implies that the AGP-free diet does not negatively impact meat yield or organ development, supporting its potential as an effective antibiotic alternative. The dressing percentage averaged 70 to 72% across all groups, results with similar findings study given *Orthosiphon stamineus* Benth leave juice in drinking water [42]. This finding is important for producers to shift to AGP-free production, as it suggests no compromise in meat yield, crucial for profitability. Similar results were reported by Hernández et al. [41], who found no differences in organ weight between control and antibiotics or plant extract treatments in 42-day-old broilers.

The increased redness in the antibiotic group was found with studies suggesting that antibiotics could affect muscle characteristics including color. This was likely due to altered muscle oxygenation or heme concentration, which was a response to improved growth and muscle [43]. The AGP-free group's intermediate redness level (1.99) suggested that AGP-free additives improve meat color by helping the microbiome balance and improving nutrient absorption. Although pH differences were expected due to the uncontrolled pre-slaughtering and slaughtering process, the antibiotic and AGP-free group tended to have a lower cooking loss (P=0.421) and greater yellowness (P=0.565) compared to control. A previous study by Lu et al. [42] found no significant differences in the meat color (lightness, redness, and yellowness), meat pH values, and cooking loss in broiler chickens. However, contrary to present results, [44] reported that antibiotic combinations increased the cooking loss and lightness while reducing the redness during the whole raring and withdrawal period. Water in meat may be attributed to capillarity with some in the myofibrils and extracellular space, and the remaining amount is situated between the myofibrils within a fiber [45]. Consequently, larger fibers exhibit to have reduced water-holding capacity, resulting in higher cooking loss, due to the larger spaces within and between its myofibrils [44]. In this experiment, broiler meat treated with antibiotics noted higher cooking loss and color properties [46]. It may be due to the growth-enhancing effect of sub-therapeutic doses which influence muscle fiber diameter, tenderness, and cooking loss with lightness (L) value in white muscles linked to drip loss and pH [47].

The performance results showed that both AGP-free and conventional antibiotic programs support similar growth outcomes in broilers. AGP-free diets supplemented with growth-promoting foods like prebiotics, probiotics, or plant extracts could promote development and improve gut health, nutrient absorption, and immune responses, mimicking the growth-enhancing effects of antibiotics [47]. This finding supported prior research indicating that AGP-free additives can serve to promote growth, achieving body weight and gain without the drawbacks of antibiotics. A study on phytogenic feed additives further explored their potential as an AGP alternative to impact broiler growth performance [48]. The comparable FCR and feed intake values between the groups suggest that AGP-free diets maintain feed efficiency critical for profitability. The small difference in FCR suggests that both AGP-free diets and antibiotics help animals utilize feed efficiently, benefiting producers looking to reduce antibiotic use without increasing feed costs. Additionally, the similar livability rates in both groups (97.45% for AGP-free vs. 97.89% for conventional) showed that AGP-free diets do not negatively impact broiler health. This finding was evidence that natural additives can boost immune systems, lower disease susceptibility [42], and improve growth performance and feed compared to AGPs [47].

The economic evaluation showed that the AGP-free approach offers significant financial advantages with lower medication costs and reduced total fixed and variable costs compared to conventional methods. However, while the AGP-free program provides more returns over variable cost and input-output ratio, it also leads to reduced broiler performance, and a rise in production expenses [42]. The AGP-free program demonstrated higher net returns (48790.80) tk and returns over variable costs (54885.66) tk compared to conventional practices (41438.94 and 47647.94) tk, respectively, reflecting this improved profitability. These results suggest that the AGP-free system reduces production costs while maximizing revenue, providing a financially appealing alternative to antibiotic use for farmers. The AGP-free program's input-output ratio of (1.36) was higher than the conventional system (1.30), indicating better cost-return efficiency, making it an economically efficient choice for broiler production. This finding with research highlights the economic benefits of drug-free practices in commercial broiler farms [49].

Conclusion

AGP-free programs for broiler chickens using prebiotics, probiotics, phytobiotics, and plant-based additives can improve growth and reduce antibiotic challenges. The study aimed to evaluate the potential of AGP-free packages as a sustainable alternative to conventional AGP use in broilers, focusing on performance matrix, blood properties, and humoral immune response. The AGP-free program, which incorporates multivitamins, acidifiers, calcium, zinc, liver tonic, and saline, led to higher body weight and weight gain in the broiler compared to both control and AGP groups. Additionally, a significant improvement in feed intake was observed in the AGP-free and AGP-treated groups than the control. The economic analysis revealed that adopting the AGP-free regimen reduced production costs by 7.50 tk per bird, suggesting economic feasibility in commercial broiler operations. The AGP-free package could effectively replace antibiotics in broiler diets without compromising productivity, providing a safer and more sustainable model for poultry production in Bangladesh. Despite these results, this study faced some limitations regarding environmental factors, management practices, and diversity of broiler operations, which may affect the consistency of outcomes when implementing the AGP-free program on a large scale. However, further study should include larger and more diverse farm samples, evaluate long-term health outcomes, and explore the effect of various AGP-free supplementations in different environmental and management contexts.

Author's contributions: This work was a collaborative effort among all authors. Author MRH carried out the planned data analysis and was responsible for writing, revising, and thoroughly reviewing the manuscript. Authors ASA and MSH contributed to the planning, structuring, writing, and revising of the manuscript. Additionally, MAGR, SS, SF, and NS assisted in rearranging, enhancing, and revising the draft. All authors carefully reviewed and approved the final version of the manuscript.

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TABLE 1. Ingredient composition in diet and its nutritional value

Ingredients (kg)	Starter (0-7 days)	Grower (8-21 days)	Finisher (22-35 days)
Maize	52.10	56.00	59.90
Protein Concentrate	9.50	9.00	7.30
Rice Polish	8.00	7.50	8.80
Soybean meal	28.00	23.80	19.80
Di Cal Phosphate	1.00	1.50	1.50
Vitamin-Mineral Premix	0.25	0.25	0.25
Salt	0.50	0.50	0.5
Oil	0.50	1.50	2.00
Lysine	0.25	0.10	0.10
Methionine	0.15	0.10	0.10
Total	100.00	100.00	100.00
ME (Kcal/Kg)	2900	3000	3100
CP (%)	23	21	19

* Contain per kg: vit. A, 12,000,000 IU; vit D3, 5,000,000IU; vitE,50,000mg; vitK3,3,000mg; vitB1, 2,000mg; vit B2, 6,000mg; vit B6, 4,000mg; vit B12, 25mg; biotin, 150mg; pantothenic acid, 20,000mg; folic acid, 2,000mg; nicotinic acid, 70,000mg. 2Contain per Kg: Fe, 66,720 mg; Cu, 41,700 mg; Mn, 83,400 mg; Zn, 66,720 mg; I, 834 mg; Se, 250 mg.

TABLE 2. The schedule for the AGP-free program for broiler production

Age of broiler	Name of medication	Doses of administration	Time of administration
1-2 days	Glucose,	50 g/L drinking water	24 hours drinking water
	Vitamin C	1-2 g/5 L drinking water	
3-6 days	Multivitamin,	1 ml/3 L drinking water	8 hours of drinking water
	Ginger juice	200-500 ml/sack feed	8 hours of drinking water
6 days	IB+ND (Live) vaccine	1 drop	One time in the eye
7-9 days	Acidifier	1 ml/1 L drinking water	8 hours of drinking water
	Calcium, Phosphorus	1 g/1 L drinking water	8 hours of drinking water
10-11 days	Zinc	1 g/1 L drinking water	8 hours of drinking water
	Vitamin ADE_3	1 ml/1 L drinking water	8 hours of drinking water
12 day	IBD vaccine	1 drop	One time in the eye
13-15 days	Liver Tonic	1 ml/2 L drinking water	8 hours of drinking water
	Essential Oil	1 ml/10 L drinking water	8 hours of drinking water
16-17 days	Multivitamin	1 ml/3 L drinking water	8 hours of drinking water
	Acidifier	1 ml/1 L drinking water	8 hours of drinking water
18 day	IBD vaccine	1 drop	One time in eye/drinking water
19-21 days	Vitamin ADE_3	1 ml/1 L drinking water	8 hours of drinking water
	Calcium, Phosphorus	1 g/1 L drinking water	8 hours of drinking water
22 day	IB+ND (Live) vaccine	1 drop	One time in eye/drinking water
22-26 days	Essential Oil	1 ml/10 L drinking water	8-16 hours drinking water
26- up to marketing	Saline	1 g/2 L drinking water	10 AM-6 PM drinking water
	Vitamin C	1-2 g/5 L drinking water	8 hours of drinking water

* Data on growth performance, i.e., body weight, weight gain, feed intake, FCR, and mortality, were kept accordingly on 7, 14, 21, and 35 days.

TABLE 3. Farmers' traditional broiler production schedule using antibiotics (group 1)

Age	Morning	Noon	Afternoon/Evening
1	Bitafin	Bitafin	Vitamin WS
2-4	Selco pH	Flumequin	Flumequin
5	Ranikhet+ Bronchitis (live)-Eye/water at night		
	B_1+B_2, Avigat	Avigat	E-cell
6-7	Avigat, B_1+B_2	Selco pH	Brawler boost, Avigat
8-9	Avigat, Selco p	Selco pH	Brawler boost, Selco pH
10	Selco pH, Calcium	Selco pH	Brawler boost, Selco pH
11	Gumboro (live)- Eye/water at night		
	Avigat, Calcium	Avigat	E-cell
12	Selco pH, Calcium	Selco pH	Selco pH
13-15	Diarrhea	Medicine	
16	Nephrosef	Bitafin, Saline	Brawler boost, Selco pH
17	Nephrosef, Avigat	Bitafin, Saline	Brawler boost, Avigat
18	Gumboro (live)- water at night		
	Nephrosef, Avigat	Bitafin, Saline	E-cell
19-20	Avigat, Selco pH	Bitafin, Saline	Brawler boost, Avigat
21	Ranikhet+ Bronchitis (live)-Eye/water at night		
	Avigat	Bitafin, Saline	E-cell
22	Liver tonic	Bitafin, Saline	Selco pH
23-24	Liver tonic	Bitafin, Saline	Brawler boost, Selco pH
25-26	Avigat, Selco pH	Bitafin, Saline	Brawler boost, Avigat
27	Zinc	Bitafin, Saline	Brawler boost, Selco pH
28	Avigat, Calcium	Bitafin, Saline	Zinc, Selco pH
29	Selco pH, Calcium	Bitafin, Saline	Zinc, Timsen
30	Calcium	Bitafin, Saline	Brawler boost, Selco pH
31	Avigat	Bitafin, Avigat	Brawler boost, Selco pH
32	Liver tonic, Avigat	Bitafin, Avigat	Brawler boost, Selco pH
33	Liver tonic, Avigat	Bitafin, Avigat	Brawler boost, Selco pH
34	Liver tonic, Avigat	Bitafin, Avigat	Brawler boost, Selco pH
35	Avigat	Bitafin, Avigat	Avigat

TABLE 4. AGP-free program for the farmers (group 2)

Age (D)	Medication	Age (D)	Medication
1	Gluco-C Vet, Vita plus WS	**17-18**	Emoboost, Gronut
1-4	Maxitin Vet, Granott, Vita plus WS	**17**	Gumboro disease vaccine
5	Ranikhet disease vaccine	**19-21**	Tileopleus, Syndro pH
5-6	Emoboost, Granott	**22-23**	E-cell biotin, Emoboost Granott
7 -9	Vitamin B_1+B_2+B_6, ADE Max Gold, Rikal Syndro pH	**23**	Ranikhat disease vaccine
10	Gumboro disease vaccine	**24-25**	Hepolov, Vitazim, ADE Max Gold, Syndo pH
10-11	Emoboost, Granott	**26-28**	Rikal, ADE Max Gold, Vita plus WS, Granott
12-14	Ajax	**29-35**	Granott, Syndro pH
15-16	Hepolov, Vitazim, ADE Max Gold, Rikal Sindo PH		

D = Day

TABLE 5. Effect of AGP-free program on the performance of broiler chicken

Parameters	Treatments			SEM	P value
	Control	AGP-free	Antibiotics		
Body weight (g)					
At 7 d	224.14	225.31	229.75	1.50	0.504
At 14 d	567.73	572.29	578.82	2.64	0.126
At 21 d	1001.55[b]	1056.89[a]	1072.43[a]	18.13	0.012
At 35 d	2260.07[b]	2319.23[a]	2349.20[a]	21.19	0.014
Weight gain (g)					
0-7 d	174.64	175.66	180.25	1.50	0.504
8-21 d	777.41[b]	831.58[b]	842.68[a]	3.68	0.008
22-35 d	1258.52[b]	1262.34[a]	1266.77[a]	10.42	0.004
0-35 d	2210.42[b]	2269.73[ab]	2299.55[a]	18.29	0.032
Feed intake (g)					
0-7 d	200.01	208.27	221.14	1.64	0.587
8-21 d	1199.25	1195.59	1202.28	8.69	0.872
22-35 d	2163.46	2155.28	2132.39	11.25	0.851
0-35 d	3562.71	3559.14	3505.81	27.53	0.214
FCR					
0-7 d	1.145	1.185	1.226	0.023	0.354
8-21 d	1.543[a]	1.437[a]	1.426[b]	0.157	0.043
22-35 d	1.719[a]	1.720[a]	1.643[b]	0.021	0.041
0-35 d	1.611	1.568	1.531	0.047	0.068

*AGP = Antibiotic growth promoter; FCR = Feed conversion ratio; SEM = Standard error of the mean.

TABLE 6. Effect of AGP-free program on blood properties of broiler chicks

Parameters	Treatments			SEM	P value
	Control	Antibiotic	AGP-free		
Different count					
Lymphocyte %	22.67	27.67	27.40	2.133	0.561
Eosinophil %	3.33	3.00	2.60	0.204	0.709
Basophil %	1.33	0.33	0.20	0.170	0.054
Red blood cells					
Haemoglobin %	9.35	10.18	10.26	0.196	0.184
ESR (mm in 1st h)	7.00[b]	11.20[a]	8.80[b]	0.597	0.047
Lipid properties					
Glucose (mg/dl)	228.72	207.68	208.30	5.78	0.314
Cholesterol(mg/dl)	182.58	149.54	194.76	2.73	0.178
HDL (mg/dl)	118.08	97.68	118.78	4.19	0.031
LDL (mg/dl)	33.16	37.14	35.36	1.93	0.172
Total protein (g/dl)	7.14	7.52	7.78	0.914	0.240
Liver enzyme activities					
ALT (U/L)	7.60	3.40	4.00	0.314	0.342
AST(U/L)	223.80	234	235.40	6.01	0.761
Humoral immune response					
IgA (µ/ml)	20.87	19.72	21.42	0.96	0.014
IgG (µ/ml)	1.80	2.25	2.18	0.08	0.021

*AGP = Antibiotic growth promoter; HDL= High-density lipoprotein; LDL = Low-density lipoprotein; ALT = Alanine aminotransferase; AST = Aspartate transaminase; SEM = The standard error of the mean; ESR = Erythrocyte Sedimentation Rate; IgA=Immunoglobulin A; IgG=Immunoglobulin G.

TABLE 7. Effect of AGP free program on carcass characteristics of broiler chicks

Parameters	Treatments			SEM	P value
	Control	AGP-free	Antibiotic		
Body weight (g)	2260.07^{b}	2319.23^{a}	2349.20^{a}	21.19	0.014
Carcass weight (g)	1582.05	1674.49	1669.57	13.59	0.587
Dressing %	70.10	72.20	71.07	2.43	0.038
Breast meat (% BW)	23.86	21.0	22.20	0.91	0.249
Thigh meat (% BW)	19.26	20.13	19.29	0.87	0.247
Drumstick (% BW)	12.56	12.26	11.60	0.69	0.359
Wing (% BW)	12.26	11.92	12.60	0.64	0.289
Liver (% BW)	2.36	2.20	2.30	0.16	0.817
Heart (% BW)	0.52	0.53	0.55	0.03	0.598
Fat pad (% BW)	2.80	2.77	2.83	0.17	0.378
Gizzard (% BW)	2.46	2.21	2.19	0.15	0.591
Spleen (% BW)	0.11	0.09	0.11	0.01	0.428
Shank (% BW)	5.06	4.18	5.08	0.38	0.241
Bursa (% BW)	0.08	0.06	0.07	0.004	0.127

*AGP = Antibiotic growth promoter; BW = Body weight; SEM = Standard error of the mean.

TABLE 8. Effect of AGP-free program on meat quality of broiler chicks

Parameters	Treatments			SEM	P value
	Control	Antibiotic	AGP-free		
Cooking loss (%)	12.01	10.65	10.30	0.58	0.421
Meat color					
L*	48.83	49.55	47.88	0.520	0.663
a*	1.64^{c}	2.68^{ab}	1.99^{bc}	0.197	0.003
b*	6.95	7.82	7.03	0.305	0.565
pH	5.69^{b}	6.05^{a}	6.05^{a}	0.013	0.030

*AGP = Antibiotic growth promoter; L = Lightness; a = redness; b = yellowness; SEM = Standard error of the mean.

TABLE 9. Growth performance of broiler at 32 days under AGP free and conventional program

Parameter	AGP-free program	Conventional program	SEM	P value
Body weight (g)	2041.61	2071.96	9.48	0.124
Weight gain (g)	1991.96	2022.31	6.61	0.487
Feed intake (g)	3259.55	3192.77	22.43	0.435
FCR	1.636	1.578	0.041	0.129
Livability (%)	97.45	97.89	1.25	0.842

* FCR= Feed conversion ratio; AGP= Antibiotic growth promoter; SEM= Standard error of the mean.

TABLE 10. Economic Returns from birds reared in AGP-free and Conventional Systems

Capital investment	AGP-free	Conventional practices
Shed price (depreciation cost) (10 %)	1800	1800
Feeder (depreciation cost) (20%)	150	150
Drinker (depreciation cost) (20 %)	58.4	58.4
Chick feeder (20% depreciation)	116.6	116.6
Chick drinker (20% depreciation)	41.6	41.6
Electric fan (depreciation cost) (20%)	160	160
Electric Bulb (depreciation cost) (20%)	150	150
Electric connection Meter, cable, socket, and accessories (5%)	80	80
Brooder	52	52
Bank interest (@5% on variable cost)	2496.17	2609.08
Land rent (Tk 300/decimal/year) (Max. 8.5 decimals)	510	510
Water tank, pipe, sprayer, bowl, etc.	80	80
Miscellaneous	400	400
Total fixed cost	6094.77	6207.68
Variable cost		
Day-old chicks' price	45000	45000
Total feed cost	124569.9	122703.35
Labor charge	6000	6000
Vaccine cost	2875	2875
Medication cost	6405	17260
Disinfectant cost	1050	1050

Capital investment	AGP-free	Conventional practices
Electricity cost	3000	3000
Litter cost (Rice husk)	1950	1950
Mortality (%)	5844.4596	5638.08
Total variable cost	197694.36	208726.43
Total cost (FC+VC)	203789.14	214934.11
Returns		
Broiler sell	272463.14	276265.92
Feed bag sell	696.87961	687.13876
Litter sell	2700	2700
Gross returns	252580.02	256373.05
Net returns	48790.8	41438.94
Return over variable cost	54885.66	47647.94
Input-output ratio	1.36	1.30
Share of fixed cost in Input-output ratio	0.04	0.04
Share of variable cost in Input-output ratio	1.32	1.26

*AGP= Antibiotic growth promoter; SEM= Standard error of the mean; FC= Fixed costs; VC= Variable costs

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