Optimization of Poultry Physiological Condition in the Post-Antibiotic Era through Nutritional Intervention – A Review

Sugiharto Sugiharto[1]*, Muhammad Asif Raza[2]
[1]Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java 50275, Indonesia, [2]Department of Pathobiology, Faculty of Veterinary and Animal Sciences, MNS University of Agriculture, Multan 66000, Pakistan.
*Corresponding author: sugiharto@lecturer.undip.ac.id

Abstract

The intestinal microbe population has a considerable impact on the physiological state of poultry. Nutritional intervention can be used to enhance the equilibrium of the gut microbiota in chickens, leading to good physiological effects. Various nutritional interventions have been implemented, including feed additives, vitamins, and fermented and functional feeds. While numerous studies have shown the efficacy of nutritional strategies, others have noted the variability of these interventions on poultry's physiological state. Various factors can influence the effectiveness of nutritional interventions on the physiological conditions of poultry, such as the types of feed additives or active components, duration and method of administration, feed composition, environmental conditions during rearing, quality of day-old-chick, type and age of poultry, hygiene, infection, stress, and other related variables. Feed additives, when combined with nutritional components or other active chemicals, can have synergistic and complementary effects. These impacts could improve the effectiveness and reliability of the nutritional interventions on physiological parameters and poultry productivity. Furthermore, combinations can include feed additives or active components like probiotics and acidifiers, probiotics and enzymes, phytobiotics and enzymes, and plant-based materials and chitosan. The combinations show synergistic and complementary benefits, improving physiological conditions in chickens more than using feed additives or active substances alone. Overall, nutritional intervention can be used to improve the microbial balance in poultry intestines and boost their physiological state. Moreover, integrating feed additives with other active components might enhance the physiological circumstances of chickens due to the synergistic effect produced by their combination.

Keywords: antibiotics, feed additives, nutritional interventions, physiological condition, poultry

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INTRODUCTION

The yearly demand for food sources containing animal protein keeps rising, aligning with population growth and expanding public awareness. Poultry products e.g. meat and eggs are an inexpensive source of animal protein with excellent nutritional value. Farmers and the poultry industry have raised the population of chicks due to the rising demand for chicken products. Data from Statistics Indonesia revealed that the overall poultry population in 2022 exceeded 3 billion birds, most of which were broiler chickens (3,114,027,615 birds) (BPS, 2022). Additionally, this population included various types of poultry, such as free-range chickens (308,601,685 birds), laying hens (379,279,968 birds), ducks (56,728,470 birds), turkeys, quail, and other avian species (BPS, 2022). Apart from increasing population, enhancing poultry productivity remains crucial in meeting the rising demand for poultry products. Productivity enhancement will encourage poultry farming towards greater efficiency, ensuring the sustained poultry industry.

Productivity theoretically signifies the aggregation of diverse physiological processes within a bird's body. A favorable correlation is present between the bird's physiological state, productivity, and health in this situation. Maximizing chicken productivity requires optimizing the physiological conditions and processes within the bird's body (Sugiharto *et al*., 2021[a]). Optimization is a process to achieve conditions that provide maximum results from a function. Optimizing physiological conditions can be interpreted as an attempt to ensure that the bird's body remains in a state of homeostasis in various environmental conditions including stress conditions such as heat stress and high stocking density-induced stress. Homeostasis is essential for good bodily function and to enhance poultry productivity (Sugiharto *et al*., 2021[a]). Recent studies have shown a connection between gut bacteria and physiological characteristics. Borda-Molina *et al*. (2018) and Aruwa *et al*. (2021) state that the microbial community in this scenario significantly influences the immune system's regulation, organ development, and metabolic processes in the bird's body. Chicken producers utilized antibiotic growth promoters (AGP) extensively in the past to control the population of harmful microorganisms in the intestines. Since January 2018, the Government of the Republic of Indonesia has completely banned the use of AGP in feed, in accordance with Minister of Agriculture Regulation No. 14/2017 on the Classification of Animal Medicines. This prohibition jeopardizes physiological condition and efficiency of poultry (Ogboko, 2011; Ismail *et al*., 2013) and enhances the number of harmful microorganisms in the intestines (Shah *et al*., 2022).

Economically, the AGP ban has significantly reduced the efficiency of poultry farming related to less efficient feed conversion, retarded growth, high morbidity and mortality rates, and increased medical costs. Researchers and poultry nutritionists have made various efforts to overcome the problems that rise after the AGP ban. Nutritional interventions are a method for optimizing physiological conditions in poultry. Common nutritional interventions in poultry include the usage of feed additives such as probiotics, synbiotics, enzymes, prebiotics, phytobiotics, antioxidants, organic acids and vitamins, as well as fermented and functional feed (Sugiharto, 2016; Gadde *et al*., 2017; Sugiharto and Ranjitkar, 2019). Previous studies reported that nutritional interventions could provide direct benefits to the microbial balance in the intestine (Sugiharto, 2016; Sugiharto and Ranjitkar, 2019) as well as indirectly contribute to the physiological condition, health and productivity of poultry (Borda-Molina *et al*., 2018; Aruwa *et al*., 2018; Aruwa *et al*., 2018; Aruwa *et al*., 2018; al., 2021).

Although many studies report the effectiveness of nutritional interventions, a few studies also report the inconsistency of nutritional interventions on the physiological condition of birds (Cheong, 2014; Sugiharto, 2016; Anadón *et al*., 2019). Numerous factors have been documented to impact the consistency of nutritional interventions on the physiological condition of birds. These factors include the type of feed additives or alternatives to AGP utilized, duration of administration, levels in the feed, method of administration via drinking water or feed, environmental conditions at the time of delivery, feed composition, quality of day-old-chick (DOC), type and age of poultry, cage hygiene, and presence of infection or stress, etc. (Sugiharto, 2016; Gadde *et al*., 2017). Based on these inconsistencies, intensive research is still required to identify an alternative replacement for AGP that not only consistently optimizes the physiological conditions of poultry but also proves cost-effective, easily storable, and field-applicable. Furthermore, the alternative should prevent harmful consumer residues while ensuring the poultry sector's long-term sustainability.

Several researchers are attempting to address the inconsistency of nutritional interventions in poultry by combining a number of feed additives with other nutritional components in an effort to achieve a breakthrough. This blend is expected to have synergistic and complementary impacts that can enhance the efficacy and consistency of dietary interventions on physiological conditions and poultry productivity (Masud *et al*., 2016; Sapsuha *et al*., 2021; Singh *et al*., 2020; Sugiharto, 2021[a]). It is, however, important to carefully consider the type of feed additives, their levels in the ration, the composition and nutrient content of the feed, chicken factors, and environmental conditions when combining products for optimal consistency and effectiveness (Sapsuha *et al*., 2021; Sugiharto, 2021[a]).

MATERIALS AND METHODS

Using the following criteria, we performed a literature search to prepare the review, concentrating on nutritional interventions to enhance the physiological state of poultry (broiler, hen, and quail): (1) English-language peer-reviewed journal articles were included; (2) books and chapters in an edited book were included with selections made; and (3) human studies were included with selections made to corroborate and bolster the data on poultry. Probiotics, prebiotics, acidifiers, enzymes, phytobiotics, vitamins, fermented feeds, physiological status, and poultry were among the search terms used in the literature. We used several scientific portals, such as Elsevier ScienceDirect, EBSCO E-journal, Proquest Research Library, Cambridge University Press E-journal, SpringerLink E-journal, and Google Scholar, to gather the relevant articles.

RESULTS AND DISCUSSION

Probiotics, Prebiotics and Synbiotics to Optimize the Physiological Conditions of Poultry

Probiotics are a type of feed additives for poultry that consist of live microorganisms. In a particular population (minimum 1×10^6 CFU per gram or per mL), probiotics can positively influence the host's health and physiological condition (Sugiharto, 2016). Various types of probiotics have been developed and applied for poultry, and the most common are probiotics based on lactic acid bacteria (LAB), yeast and *Bacillus* strain (Isroli *et al*., 2017; Sugiharto *et al*., 2018[a]; Sugiharto *et al*., 2018[b]; Sugiharto *et al*., 2018[c]; Sugiharto *et al*., 2019[a]; Mangisah *et al*., 2021; Astuti *et al*., 2022; Sugiharto *et al*., 2022[a]). Several researchers have also begun to develop fungus-based probiotics for poultry (Sugiharto *et al*., 2015; Sugiharto *et al*., 2018[d]). Although the precise mechanism by which probiotic bacteria and yeast improve poultry physiological condition is still being researched, several probable actions are believed to contribute to their positive impact on avian physiological condition, health, and production. These mechanisms include the following: (1) Preserving the balance of beneficial bacteria in the intestine through competitive exclusion activities (pathogenic bacteria compete with beneficial bacteria for nutrients in the intestinal mucosa) and antagonism (pathogenic bacteria are inhibited from growing by producing 'toxins' like lactic acid); (2) Promoting intestinal integrity and maturation; and (3) Regulating the immune system and reducing inflammation; (4) Enhancing metabolic processes through the production of less ammonia and more digestive enzyme activity; (5) Boosting feed consumption and digestion (due to an improvement in the microbial balance in the gut); and (6) Stimulating the immune system and neutralizing enterotoxins. Probiotics derived from fungi typically function similarly to those derived from bacteria or yeast in terms of their mode of action. Fungal probiotics have antimicrobial activity and can modulate intestinal microbiota, stimulate digestive enzyme activity, and reduce cholesterol concentrations in the poultry (Sugiharto, 2019; Agustono *et al*., 2024).

The poultry digestive organs cannot digest prebiotics but are very useful for the life of beneficial bacteria in the digestive tract. Prebiotics are often identified as a food source for commensal bacteria, such as *bifidobacteria* and *lactobacilli* in the intestine. In this scenario, prebiotics offer more incredible benefits than probiotics as they stimulate the growth of good bacteria well adapted to the poultry gut environment (Sugiharto, 2016). Currently, prebiotics that is widely used for poultry include fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS), and mannan oligosaccharides (MOS). Previously, it was reported that the exact mechanism by which prebiotics influence the physiological condition of the host remains uncertain Sugiharto (2016) and Ricke (2018). Prebiotics' ability to boost the amount of LAB in the gut, however, is thought to support competitive exclusion efforts against pathogens from the poultry digestive system. Reducing the number of pathogenic microbes in the chicken gut can also be accomplished by increasing the production of short-chain fatty acids (SCFA) by giving prebiotics, which raises intestinal acidity (Fikri *et al*., 2024). Additionally, prebiotics have been shown to boost immunity, which will help the chicken fight off invasive pathogens. Prebiotics' potential to boost immune response may include direct interactions between prebiotics and immune cells in the intestine. Prebiotics may also influence the development of immune cells and intestinal tissue indirectly by boosting the number of advantageous microorganisms in the intestines. In this context, prebiotics function similarly to probiotics to safeguard chicken intestinal health (Sugiharto, 2016).

Prebiotics and probiotics are frequently used in combination to boost the effectiveness in enhancing the physiological parameters, health, and productivity of poultry. Previous research has shown that synbiotics (a blend of inulin extracted from *Dioscorea esculenta* L. and the probiotic *L. plantarum*) enhance broiler growth rate, protein digestibility, and gut ecology (Setyaningrum *et al*., 2019). Another work conducted by Sunu *et al*. (2021) reported that the use of synbiotics derived from garlic extract and the probiotic *Lactobacillus acidophilus* can improve the blood profile and antioxidant status of broilers. Additionally, in comparison to the control group, this combination enhanced the small intestine's microbial population and morphology, which enhanced the growth performance of broilers.

Generally, the mechanism by which synbiotics enhance the physiological condition of poultry involves a synergistic combination of the two active components of probiotics and prebiotics (Sugiharto, 2016). In particular, when compared with the use of probiotics and prebiotics individually, synbiotics have been reported to show better results. From physiological parameters, Abdel-Fattah and Fararh (2009) reported that the administration of synbiotics can further increase the levels of total protein, albumin, and globulin when compared to the administration of probiotics or prebiotics alone. It is thought that prebiotics and probiotics have complementary and synergistic effects that can further boost the efficacy of synbiotics in enhancing the physiological state of chickens.

Regarding the efficacy of synbiotics in maintaining the physiological condition of poultry, the administration of synbiotics increased the number of erythrocytes, hemoglobin concentration, total protein concentration in plasma, number of leukocytes and reduced the ratio of heterophils to lymphocytes (Mangisah *et al*., 2022). Generally, an increase in erythrocyte and hemoglobin values is associated with an increase in the rate of energy metabolism and poultry growth (Astuti *et al*., 2022). The rise in overall plasma protein levels in this study suggests enhanced digestibility and utilisation of nutrients, particularly protein derived from diet. Enhanced protein digestion and absorption are facilitated by improvements in ecology and intestinal function with the injection of synbiotics (Sugiharto, 2016). Furthermore, Mangisah *et al*. (2022) reported that synbiotics can lessen the potential adverse effects of stress in chickens. This was demonstrated by a reduced ratio of heterophils to lymphocytes (H/L) in broiler chickens provided with synbiotics. The low potential for stress improves the immune function of chickens given synbiotics, as seen from the increase in the number of leukocytes and the relative weight of the *bursa of Fabricius* and thymus in chickens. Regarding the ability of synbiotics to prevent stress in chickens, Mounir *et al*. (2022) reported that synbiotics have a high polyphenol content to improve antioxidant status and prevent oxidative stress conditions in the host. However, it should be remembered that the polyphenol content in synbiotics varies greatly depending on the probiotic microbes and types of prebiotics used as synbiotics.

Organic Acids to Optimize the Physiological Condition of Poultry

Amino acids and fatty acids with the R-COOH structure are examples of carboxylic acids, which make up organic acids. Poultry farmers now frequently employ organic acids as an alternative to AGP following Indonesia's ban on its use in livestock production. Propionic acid, formic acid, citric acid, and acetic acid are common organic acids substituted for AGP in poultry farming. Organic acids have been identified as antimicrobial agents for poultry in the majority of literature. According to Khan *et al*. (2022), it has to do with the capacity of organic acids to reduce intestinal pH in order to prevent the growth of pathogenic bacteria, the majority of which are less tolerant of an acidic pH. Apart from improving growth and nutrient digestibility, organic acids have also been reported to improve intestinal integrity and morphology, immune response, and physiological conditions of poultry (Sugiharto, 2016; Khan *et al*., 2022).

Isroli *et al.* (2020) investigated the effect of organic acids as feed additives, specifically formic acid, butyric acid, or a combination of the two, on broiler chicken growth rate, haematological indices, and intestinal morphometrics. From a physiological perspective, using organic acids (formic acid, butyric acid, or a combination) has been associated with decreased platelet counts in broiler chicken blood (Isroli *et al*., 2020).

The administration of organic acids, especially butyrate, was reported to increase the albumin concentration in the serum of broiler chickens. Supplementation of butyric acid can improve nutrient digestibility, especially protein, thereby increasing the concentration of albumin (Imran *et al*., 2018). Leeson *et al*. (2005) further explained that butyric acid can improve intestinal morphology, enhancing protein absorption. The results mentioned above were further confirmed by Isroli *et al*. (2020), who reported an increase in villi height in broiler chickens that received butyric acid.

Phytobiotics to Optimize the Physiological Condition of Poultry

Medicinal plants have been used for therapeutic purposes against a variety of diseases since the dawn of human civilization. It is among the earliest known methods of providing healthcare to humans. Apart from human application, herbal ingredients, known as phytobiotics, are also commonly used for poultry. Besides stimulating appetite and the secretion of endogenous enzymes, phytobiotics exhibit antimicrobial, coccidiostatic, and anthelmintic activities in poultry (Sugiharto, 2021[b]). Along with traditional home production, many modern pharmaceutical industries in Indonesia are currently active in manufacturing and marketing phytobiotics for livestock, particularly poultry (Medion, 2019). Indeed, herbal ingredients as feed additives are trendy for use in poultry following the ban on AGP in Indonesia. Previously, it was stated that phytobiotics can improve the hematological profile by increasing hemoglobin and hematocrit values, as well as mean corpuscular volume (MCV), thereby maximizing the growth capacity in poultry (Sugiharto, 2021[b]). In addition, phytobiotics contain various phenolic components to improve antioxidant status and reduce the deleterious impact of stress on poultry (Sugiharto, 2016; Sugiharto, 2021[b]).

Stress can cause oxidative damage and physiological abnormalities in poultry. A study was done to investigate the impact of encapsulated *Cosmos caudatus* leaf extract on the health and antioxidant levels of broilers grown in stressful crowded pens. The study demonstrated that encapsulated *C. caudatus* leaf extract increased the concentration of superoxide dismutase (SOD) enzyme in chicken serum (Agusetyaningsih *et al*., 2022). These results confirm the capacity of phytobiotics to improve the antioxidant status of birds, especially under stress circumstances. Moreover, phenolic components in phytobiotics have a dominant role in enhancing the antioxidant status of poultry (Sugiharto, 2021[b]). Another study further reported the positive impacts of *C. caudatus* leaf extract on the immune function of broiler. They specifically showed that *C. caudatus* leaf extract could protect the *bursa of Fabricius* of broiler chickens from stress-induced damage. In this case, the flavonoids in *C. caudatus* leaves can prevent damage to cells and lymphoid tissue due to excessive production of free radicals when chickens experience stress. Also, herbal products can increase chicken immunity by promoting the lymphocyte cells proliferation in poultry immune organs that experience immunosuppression due to oxidative stress (Agusetyaningsih *et al*., 2022). Additionally, supplementation of acidified turmeric flour (using *Averrhoa bilimbi* L. filtrate as a source of acid) could improve the antioxidant capacity of the broiler by enhancing the SOD and CAT enzyme activities, and decreasing the MDA (Sugiharto, 2021[b]).

Fermented Feed to Optimize the Physiological Conditions of Poultry

Fermentation is a simple technique commonly used to improve the chemical composition and functional value of poultry feed stuffs (Sugiharto and Ranjitkar, 2019). Moreover, fermentation can enhance the antioxidant potential of feed ingredients (Sugiharto *et al*., 2019). Fermentation can also elevate the amount of LAB, lactic acid, and other acids in poultry feed ingredients (Sugiharto and Ranjitkar, 2019). Generally, fermentation can be grouped into solid-state (SSF) and submerged fermentation (SmF). In comparison to SmF, SSF is more widely applied in chicken feed production because SSF results in products with more favour characteristics (Sugiharto *et al*., 2019[b]).

Based on the content of various active components in fermented feed ingredients, Sugiharto *et al*. (2017[a]) carried out a nutritional intervention using fermented feed ingredients (fermented cassava pulp using filamentous fungus *Acremonium charticola*) to optimize the physiological conditions of broilers. The data showed that feeding fermented feed increases the production of SCFA, especially butyric acid, in the cecum, thereby potentially improving ecosystem conditions and poultry gut health. The high content of LAB in fermented feed ingredients has been reported to increase the counts of anaerobic bacteria, which are responsible for the production of propionic and butyric acids in the caecum of broiler chickens (Sugiharto *et al*., 2017[a]; Sugiharto and Ranjitkar, 2019). Providing fermented feed can also prevent chickens from infections, especially viruses, which is indicated by a lower percentage of lymphocytes in the blood and globulins in the serum. In this case, the active components in fermented feed ingredients especially LAB, organic acids and antioxidants are responsible for improving the immune response to minimize the invasion of pathogens, especially viruses, which enter the body of broiler chickens (Sugiharto and Ranjitkar, 2019).

In another study, Sugiharto *et al*. (2017[b]) reported that using fermented ingredients (*A. charticola*-fermented cassava pulp) improved the antioxidant status of broiler chickens. In this case, fermented feed can prevent oxidative stress in the body of chickens so that the level of 2,2-diphenyl-1-picrylhydrazyl (DPPH; % inhibition) is lower in the group of chickens given fermented cassava pulp when compared with the control group. The antioxidant capacity of the fungus *A. charticola* (Sugiharto *et al*., 2016[a]), which is used to ferment cassava pulp, is very likely to be able to reduce oxidative stress in chickens (Sugiharto *et al*., 2017[b]; Sugiharto *et al*., 2019[a]; Sugiharto *et al*., 2019[b]) which is characterized by a low DPPH percentage inhibition in broiler chickens. Of note, antioxidant activity will increase in response to the elevated production of free radicals which cause oxidative stress in broiler chickens (Sugiharto *et al*., 2017[b]). Other studies reporting the beneficial effects of fermented feeds on the physiological conditions of some poultry species are presented in Table 1.

Collectively, this study acts as a fundamental reference to encourage farmers to ferment feed ingredients, especially unconventional feed ingredients obtained from agricultural industry byproducts and include them in poultry rations. These components not only provide nutrition but may also exhibit a good influence on the physiological status of chickens.

Functional Feed to Optimize the Physiological Condition of Poultry

Functional feed refers to feed or ingredients that offer extra health advantages for livestock besides providing basic nutritional requirements. Apart from carbohydrates, fats, proteins, vitamins, and minerals, functional feed ingredients generally contain several active components, including terpenoids, flavonoids, capsaicinoids, carotenoids, lignin, chlorophyll, stilbene, phenolic acids, fiber, polysaccharides sterols, and others (Sugiharto *et al*., 2018[e]). These active components can be naturally contained in feed ingredients or formed due to specific processes, for example, fermentation. Using functional feed ingredients has been proven to increase chicken farmers' profits. Farmers can reduce their dependency on feed additives or supplements by incorporating these ingredients into the diet while promoting physiological conditions, health, and poultry production.

Table 1. Effects of fermented feed on poultry physiological conditions

Feedstuffs	Fermentation starter	Phyisological effects	Poultry species	References
Four parts corn mixed with 3 parts cottonseed meal and 3 parts rapeseed meal	*Lactobacillus plantarum* (CICC20765) and *L. acidophilus* (CICC6006)	Improved immune function and antioxidant capacity	Laying hens	Zhu *et al*. (2020)
63.61% corn, 25.49% soybean meal, 3.44% corn gluten meal, 3.44% corn dried distillers grains soluble and 4.02% wheat bran	*L. plantarum*, *Bacillus subtilis* and *Saccharomyces cerevisiae*	Improved immune organ development and composition of the cecal microbiota	Broiler chickens	Zhu *et al*. (2023)
Pearl millet grains	Not mentioned	Improved weight of *bursa of Fabricius* and duodenum villus width	Broiler chickens	Olasehinde and Aderemi (2023)
Not mentioned	Yeast culture	Reduced the severity of necrosis of villi in the jejunum, improved antioxidant status (decreased malondialdehyde) and lower plasma cholesterol	Nicobari chickens	Nidamanuri *et al*. (2022)
Rapeseed meal	Not mentioned	Increase duodenal villus height, superoxide dismutase and total antioxidant capacity, while decreasing malondialdehyde in serum	Broiler chickens	Wu *et al*. (2022)
Soybean meal	*L. plantarum* (PTCC1058), *B. subtilis* (PTCC1156) and *Aspergillus oryzae* (PTCC5163)	Improved gut microbiota, small intestinal morphology, and serum lipid profile	Japanese quails	Jazi *et al*. (2018)
Medicago sativa (plus soybean and distillers dried grains with solubles)	Not mentioned	Improved immunity, antioxidant status and ileum morphology	Geese	Li *et al*. (2022)

Previously, fermented dried cassava was used as a functional feed ingredient to examine the impact of dried cassava on the profile of blood in broilers. The results documented that fermented, dried cassava in feed can alleviate stress conditions in broilers, as evidenced by a decrease in the ratio of heterophils and lymphocytes (H/L ratio) in the blood of broilers

(Abdurrahman *et al*., 2022; Chandra *et al*., 2022; Faiqoh *et al*., 2023). The antioxidant capability of fermented dried cassava has been suggested to boost antioxidant status, potentially alleviating stress conditions in chickens. Moreover, fermented dried cassava also significantly reduced the triglyceride concentration in the blood of broilers (Sugiharto *et al*., 2015). This can be explained by the probiotic and antioxidant potential of the fungus that grows on fermented dried cassava. Additionally, it is suggested that the probiotic and antioxidant activities can potentially reduce fat accumulation in broilers (Sugiharto *et al*., 2016[b]). Probiotics may have a hypolipidemic effect in broilers by reducing cholesterol synthesis or by increasing cholesterol breakdown and excretion. Also, probiotics can lower blood cholesterol by deconjugating bile salts (Jafarpour *et al*., 2019). With regard particularly to antioxidants, antioxidants lower the percentage of abdominal fat in chickens by increasing the activity of hormone-sensitive lipase (HSL) in the fat and suppressing the activities of lipoprotein lipase (LPL) in the fat, malate dehydrogenase (MDH), and glucose-6-phosphate dehydrogenase (G-6-PDH) (lipogenic enzymes) in the liver (Fouad and El-Senousey, 2014; Lokapirnasari *et al*., 2022).

Another work demonstrated that using fermented banana peel as a functional feed stuff and an energy source can reduce uric acid concentration in broiler chickens' serum. Because uric acid is the byproduct of protein catabolism, broiler chickens fed fermented banana peels exhibit lower levels of protein degradation as evidenced by their lower serum uric acid concentrations. Moreover, fermented banana peel improves protein deposition potential and heightens ileal villi in broiler chickens. This increased villi height improves nutrient absorption capacity in the gut (Sugiharto *et al*., 2020).

The functional feed contains various active components that can act as antibacterials, antioxidants, and immunomodulators (Sugiharto *et al*., 2018[e]). Functional feed ingredients may improve gut ecology by enhancing microbial balance in the gut. This may have contributed to the increased height of intestinal villi observed in treated chickens compared to control chickens. It is hoped that this study will inspire poultry farmers to use unconventional feed ingredients as functional feed ingredients, as has been done with banana peels.

Enzymes to Optimize the Physiological Condition of Poultry

Enzymes are proteins that function as catalysts, influencing biological reaction rates in the body without altering themselves. Endogenous enzymes are enzymes that originate naturally within the body of each animal. For example, endogenous enzymes are digestive enzymes produced by the pancreas, stomach and small intestine of poultry (Sugiharto *et al*., 2021[a]). Although, endogenous enzymes produced naturally may not meet the body's biochemical requirements. In these conditions, exogenous enzymes are needed to increase the physiological activities in the bird's body. Various exogenous enzymes have been applied to poultry feed, including xylanase, β-glucanase, amylase, protease, α-galactosidase, lipase, and phytase to improve digestive activity. The applications of exogenous enzymes is essential in chickens, considering that poultry feed ingredients mainly consist of maize and soybean meal, which contain anti-nutritional components for instance, protease inhibitors and non-starch polysaccharides which can inhibit the digestion and absorption in the poultry digestive tract. In this case, exogenous enzymes are expected to compensate for endogenous enzyme deficiencies by assisting in the chemical digestion of feed materials or hydrolyzing specific anti-nutritional components contained in the feed (Sugiharto, 2016).

Apart from playing a role in the digestive process, using exogenous enzymes has been noticed to modify the intestinal microorganisms, improve gut morphology, and increase the immune response of birds (Sugiharto, 2016). As part of a nutritional intervention, the inclusion of enzymes in feed has been reported to improve the blood protein profile of chickens. Javaid *et al*. (2022) documented that the use of commercial multienzymes and multienzymes produced by *B.*

subtilis KT004404 could reduce the levels of uric acid. Conversely, it raises the levels of albumin, globulin, and total protein in the blood of broiler chickens. According to the researcher, adding exogenous multienzymes to feed can boost the anabolism of proteins. Purines are created in poultry by excess amino nitrogen (Pratama *et al*., 2021; Lovela *et al*., 2023). Purines are broken down by the body into uric acid, which is then eliminated. Exogenous enzymes have the ability to boost protein anabolism and inhibit overabundance of amino nitrogen (Javaid *et al*., 2022).

In broilers, the inclusion of exogenous enzymes in the feed resulted in higher hemoglobin levels, leukocyte counts, and mean corpuscular hemoglobin (MCH) and concentrations. Additionally, Sugiharto *et al*. (2023[a]) found that the use of multienzymes in the feed positively lowers the concentrations of uric acid in the serum of broiler chickens. It is well known that multienzymes improve broiler nutrition digestibility (Sugiharto, 2016), increasing nutrient availability for growth-promoting energy metabolism. Given how hemoglobin supports the metabolic processes of poultry, this could indicate that there is a greater supply of oxygen available to cells, which could support the metabolic processes of chicken cells (Wardiana *et al* ., 2021; Utomo *et al*., 2022).

In terms of the positive influence of multienzymes on increasing the number of leukocytes, multienzymes can support the development of the *bursa of Fabricius* and increase the production of leukocytes. On the other hand, exogenous enzymes can also increase the availability of nutrients needed for the production of immune cells by poultry. Using exogenous enzymes can reduce uric acid concentration in broiler' serum (Javaid *et al*., 2022; Sugiharto *et al*., 2023[a]). This condition shows the positive influence of exogenous enzymes on increasing protein anabolism and reducing muscle proteolysis. The efficacy of exogenous enzymes used as a feed additive is considered to be very beneficial for poultry farmers. This could be helpful to improve the feed digestibility, physiological condition, and health of poultry.

Inconsistency of Nutritive Interventions on the Physiological Condition of Poultry

As described above, various studies highlight the positive impact of nutritional interventions on the physiological condition of birds. However, some studies have reported the inconsistency of nutritional interventions on the physiological condition of birds (Cheong, 2014; Sugiharto, 2016; Anadón *et al*., 2019). Prior studies have demonstrated that adding formic acid and *Saccharomyces cerevisiae*, or both, to broiler chicken feed improved feed efficiency and growth performance. In order to maximize the advantages of giving formic acid or *S. cerevisiae* to broilers individually in the post-antibiotic era, a combination of the two was therefore necessary (Sugiharto *et al*., 2019[c]). Several conditions have been noted to affect the effectiveness of nutritional interventions on the physiological condition of birds, including the type of feed additives or alternatives to AGP used, duration of administration, levels in the feed, method of administration, environmental conditions, feed composition, quality of day-old chicks used, type and age of birds, hygiene, presence of infection or stress, etc. (Sugiharto, 2016; Gadde *et al*., 2017). Each type of feed additive has a specific capacity and mechanism to influence the physiological condition of birds. Several additives such as prebiotics, probiotics, synbiotics, and organic acids directly influence the ecological and microbiological conditions of the poultry digestive tract and indirectly impact the haematological condition and antioxidant status of poultry. Other feed additives, such as phytobiotics contain very high phenolic components, so they directly impact the antioxidant status and immune system of birds, especially under stress conditions (Sugiharto, 2016). Environmental conditions during rearing are a crucial factor in influencing the effectiveness of nutritional interventions on the physiological condition of birds. Environmental stress can certainly increase the production of catabolic adrenal hormones. Immunosuppression is also frequently found in birds experiencing environmental stress. This will hinder nutritive intervention activities, especially the use of enzymes, which are expected to increase the rate of energy metabolism and biosynthesis processes in the bird's body (Sugiharto, 2020[a]).

In terms of dosage, certain feed additives, particularly those based on phytobiotics, may, if added in excess, have a detrimental impact on the physiological status of birds. When encapsulated *C. caudatus* leaf extract was used at a dose of 1.5 g/kg feed, Agustianinsih *et al*. (2022) discovered that this may result in haemolytic anaemia, which could impair broiler chickens' health and physiological state. Furthermore, using lower doses of encapsulated *C. caudatus* leaf extract improves the chickens' ability to combat free radicals. Nevertheless, high dosages of encapsulated C. caudatus leaf extract may demonstrate pro-oxidant characteristics, which could cause oxidative damage to the bird's body. The types of poultry used in research are also very likely to provide different physiological responses to nutritional interventions. In contrast to the use of formic acid and *S. cerevisiae* as feed additives in broiler chickens which gave positive results, Sugiharto *et al*. (2019[c]) did not find a positive response to the use of these two feed additives in the Indonesian crossbred chickens. Differences in physiological responses, nutritional needs, tolerance to stress, immune system and organ development between broiler chickens and Indonesian crossbred chickens or other types of poultry are very likely to influence the capacity and effectiveness of nutritional interventions to optimize the physiological condition of birds.

So far, nutritional interventions to optimize the physiological condition of poultry are still inconsistent. Therefore, extensive research is still needed to find nutritional intervention methods that consistently improve the physiological condition of poultry, affordable, easy to apply in the field, and safe for consumers of poultry products. This will encourage the sustainability of poultry farming in the future.

Combination of Feed Additives with Other Nutrition Components to Optimize the Physiological Condition of Poultry

The inconsistency of nutritional interventions to improve the physiological condition of poultry is the main obstacle to implementing this method at the farmer level or in the modern poultry farming industry after the AGP ban. Several researchers and poultry nutritionists are trying to make breakthroughs by combining several feed additives with other nutritional components or active ingredients to overcome this condition. These researchers believe that the combination of several feed additives or active ingredients will create synergistic and/or complementary impact so that they can further increase the effectiveness and consistency of nutritional interventions on physiological conditions and poultry productivity (Masud *et al*., 2016; Sapsuha *et al*., 2021; Singh *et al*., 2020; Sugiharto, 2021[a]). From the productivity aspect, Masud *et al*. (2016) revealed that using blends of probiotics and acidifiers resulted in higher body weights in broiler chickens compared to control chickens or chickens given probiotics or acidifiers individually. From an economic perspective, Singh *et al*. (2020) showed that the combined use of phytobiotics and enzymes can reduce production costs and increase profits for broiler chicken producers compared to using phytobiotics or enzymes alone.

From a physiological aspect point of view, the use of a combination of probiotics (a mixture of *Streptococcus thermophilus*, *L. plantarum*, *Streptococcus feacium*, *Lactobacillus johnsonii*, *L. acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium bifidum*, *Candida pentolopsy* and *Aspergillus ourozai*) and phytase enzyme is documented to improve the cholesterol profile, liver condition and antioxidant status of broilers (Derakhshan *et al*., 2023). They further explained that the synergist between probiotics and enzymes is responsible for better chicken physiological responses. Other researchers combined probiotics (a mixture of *L. fermentum* P2, *L. acidophilus* LAP5, *L. casei* L21 and *Pediococcus acidophilus* LS) and herbs (*Gardeniae fructus*) for broilers

(Chang *et al*., 2019). They suggested that combining probiotics and phytobiotics could further increase SCFA production and improve the small intestinal morphology of chickens when compared with chickens given probiotics or herbal products alone. Another study reported that the phytobiotics (papaya seed sprouts) and chitosan were beneficial in improving nutrient bioavailability, immune responses, intestinal morphology, and meat quality of broilers at grower phase (Sugiharto *et al*., 2022[b]).

Numerous nutritional components or feed additives are combined to produce a synergistic impact. It's important to note that fermentation might increase an ingredient's concentration of active or useful ingredients (Sugiharto, 2019; Sugiharto and Ranjitkar, 2019). Previous research evaluated the effects of supplementation of *Lactobacillus casei*-fermented mixture of red rice and aromatic ginger (FERMIX) on performance, complete blood counts, gut bacterial counts, and morphological indices of broilers. The results revealed that dietary FERMIX improved broiler immune competencies, notably at 0.5%, as indicated by greater spleen weight. FERMIX at 0.5% of diets also improved physiological conditions as demonstrated by increased plasma total protein, hemoglobin, packed cell volume, and erythrocyte levels. By enhancing the duodenal VH/CD, decreasing the depth of the jejunal crypt, and lowering the counts of lactose-negative *Enterobacteriaceae*, the dietary intervention also enhanced intestinal health (Astuti *et al*., 2022).

The effect of blends of functional feed ingredients and feed additives on the physiological responses of birds has also been studied by Sugiharto *et al*. (2023[a]). The data exhibited that the combination of unripe banana flour (as a functional feed ingredient) and multienzymes led to higher values of haemoglobin, MCH, MCHC, and the ratio of villus height and duodenal crypt depth compared to those measured in chickens fed unripe banana flour alone. The combined use of raw banana flour and multienzyme also resulted in higher leukocyte and LAB counts and lower uric acid levels in the serum and duodenal crypt depth of broilers compared to controls (Agustono *et al*., 2019). This result is interesting because the administration of unripe banana flour alone did not significantly impact the abovementioned parameters. It shows the complementary effect of multienzymes on unripe banana flour, especially on improving the immune response, intestinal microbial balance, protein metabolism, and intestinal morphology of broilers.

The study conducted by Sugiharto *et al*. (2023[a]) indicated that the effects of combining unripe banana flour with multienzyme differed from those of combining unripe banana flour with probiotics on a number of parameters, including the depth of duodenal crypts, leukocyte count, uric acid concentration, and haemoglobin concentration. Probiotics did not exhibit a complementary effect to unripe banana flour as multienzymes based on the previously mentioned parameters. In comparison to chickens that received unripe banana flour alone, those that received a combination of unripe banana flour and probiotics showed no differences in haemoglobin concentration, leukocyte count, uric acid concentration, or duodenal crypt depth. The absence of synergistic and complementary effects was also reported by Sugiharto *et al*. (2019[c]), when combining formic acid and *S. cerevisiae* probiotics. The findings showed no difference between the combined use of formic acid and *S. cerevisiae* compared with the use of formic acid or *S. cerevisiae* individually in terms of SOD and MDA concentrations, immune response and serum biochemistry in Indonesian crossbred chickens. Based on the existing conditions mentioned above. Previous research provides the opinion that the consistency and effectiveness of the combination of feed additives to optimize the physiological condition of poultry is greatly influenced by the type of feed additives combined, the level in the ration, the composition and nutrient content of the feed, chicken factors and environmental conditions at the time of the study (Sugiharto, 2021[a]; Sapsuha *et al*., 2021).

The Future of Combinations of Feed Additives with Other Nutrition Components for Optimizing the Physiological Condition of Poultry Post AGP Ban

The efficacy of nutritional interventions in improving the physiological condition of poultry is highly dependent on the feed additives or active ingredients used in combination (Sugiharto, 2021[a]; Sapsuha *et al*., 2021). Currently, many researchers are paying great attention to finding the right combination of feed additives to create maximum synergistic and complementary effects to improve the physiological condition of poultry. The combined impact of chestnut extract and quebracho tannins has been evaluated, and results showed that it improves intestinal conditions and growth performance of broiler chickens (Redondo *et al*., 2022). A study also reported that a combination of citric acid and *Spirulina platensis* has beneficial effects on the physiological condition of broiler chickens (Ismita *et al*., 2022). The combined use of *Spirulina platensis* and *Saccharomyces cerevisiae* on Indonesian crossbred chickens (ICC) was reported. The results revealed that dietary supplementation of blend of *S. platensis* and *S. cerevisiae* more improved growth performance and bacterial population of the ICC (Sugiharto *et al*., 2022[a]). The antibacterial properties of *S. platensis* and *S. cerevisiae* appear to be complementary, which may have a greater impact on lowering the numbers of harmful bacteria in the intestines of chickens. Intestinal functions of the ICC were further enhanced by the latter state. Moreover, compared to individual application, the mixture of neem leaf flour (*Azadirachta indica*) and cinnamon oil (*Cinnamomum zeylanicum*) more improved the productivity of broiler chickens (Nath *et al*., 2023). In such case, both neem leaf and cinnamon oil posses digestive stimulatory agents, which synergistically improved the digestive process and nutrient utilisation of the chickens. Almeida *et al*. (2021) applied a combination of α-monolaurin mono-, di- and triglyceride butyric acid and lysolecithin as an alternative replacement for AGP. The findings revealed the improvement in antioxidant status (reduced levels of reactive oxygen species and thiobarbituric acid reactive substances [TBARS]) and productivity of broiler chickens. Additionally, Santos *et al*. (2019) reported the beneficial effects of the combination of medium-chain fatty acids (MCFA), organic acids, and polyphenols as a nutritional intervention method for broiler chickens. They revealed that the combination of MCFA, organic acids and polyphenols was effective in alleviating the negative effects of heat stress on the intestinal morphology of broiler chickens. Likewise, the feed additive combination was capable of ameliorating the oxidative stress and inflammation in the broiler intestine during heat stress (Santos *et al*., 2019).

Apart from the studies above, there are still many studies carried out by researchers to formulate the right combination of feed additives and other active ingredients in order to optimize the physiological conditions of poultry. Among these researchers are Giannenas *et al*. (2014[a]) which combines benzoic acid and essential oils. They reported that the combined product was more superior in improving the growth of turkey poults and intestinal integrity and gut microbiota. Other study by Giannenas *et al*. (2014[b]) combined benzoic acid, essential oils and protease enzymes, and found that the feed additive blend was more effective in improving weight gain, feed conversion ratio, villus height, LAB counts, and reduced coliform counts. Moreover, Nahak *et al*. (2021) combined probiotics and phytobiotics, and reported more beneficial effect of the combined additive on broiler weight gain and feed conversion as compared to control. Wang *et al*. (2021) also combined probiotics and phytobiotics. The latter investigators found that the blend of probiotics and phytobiotics strengthened the immune system, modified intestinal flora, and improved growth performance in chicks infected with *S. pullorum*. Swaggerty *et al*. (2022) combined microencapsulation of organic acids and herbal products, and showed the protective effects of the blend on the clinical signs of necrotic enteritis (due to *Clostridium perfringens*) through specific signaling pathways in broilers. Furthermore, Ebeid *et al*. (2021) combined probiotics and organic acids, and demonstrated that the additive blend improved humoral- and cell-mediated immune response and reduced *E. coli* and *Salmonella* counts in the gut. Currently, the search for the ideal combination of feed additives and other active components for poultry is being conducted not just by researchers at universities and research organizations but also by poultry nutritionists employed by private enterprises.

CONCLUSION

Nutritional intervention is a method that can be used to improve the balance of microbes in the intestine so that it positively impacts the physiological condition of poultry after the ban on AGP for livestock. Combining feed additives with nutritional components or other active ingredients is an effective nutritional intervention method after the AGP ban because it can further optimize the physiological conditions of birds due to the synergistic and complementary effects created by combining these ingredients.

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AUTHORS' CONTRIBUTIONS

SS: conceptualization and drafted the article, and MAR: revised the manuscript. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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