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THE EFFECT OF SOME MICROORGANISMS IN GASTRO-INTESTINAL TRACTS ON THE NUTRITIVE VALUE OF BROILER DIETS

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Abstract

A 2x2 factorial experiment was carried out to determine the effects of two levels of diet supplemented with and without microorganisms in combination with and without sterilized feed on the nutritive value of broiler diets with four replicates in each treatment. Some microorganisms from the gastrointestinal tract of chicken were supplemented in commercial broiler diets. They were bacterial (BC-NA-01), actinomycetes(BI-NA-03, BC-NA-02 and BL-NA-02), Aspergillus niger sp.(BD-PDA-01), Mucor sp.(BL-PDA-02), Rhizopus stolonifer sp.(BI-PDA-02) and Trichoderma sp.(BL-PDA-02). The results of proximate analysis revealed that a diet supplemented with microorganisms had a lower percentage of dry matter and crude fiber in the starter diet(0-3 wks), grower diet(4-5wks) and finisher diet(last period) than the diet without microorganisms ($p<0.05$). They were higher in the percentage of phosphorus in the starter diet and calcium in both the grower diet and finisher diet than the diet without microorganisms ($p<0.05$). The sterilized diet had a higher percentage of moisture than the non-sterilized diet ($p<0.01$). Also, they were higher in the percentage of crude protein and ether extract in the starter diet and crude fiber, calcium in both the grower diet and finisher diet than the non-sterilized diet ($p<0.05$). The diet supplemented with microorganisms and sterilized was higher in crude protein, ether extract, crude fiber, ash and metabolizable energy than the others in the starter diet ($P<0.01$). However, crude protein, and metabolizable energy were not significantly different with the control. Also, the crude protein, ash and metabolizable energy were higher than the others except the control in grower and finisher diet ($p<0.01$).

Key words: *microorganisms, gastro-intestinal tracts, nutritive value, broiler diets.*

INTRODUCTION

The chicken's gastro-intestinal tract contains approximately 40 species of microorganisms with more than three different types. They plays an important role to enhance nutrient absorption and improve growth performance, feed efficiency, and in reducing mortality from enteric pathogens (Larbier and Leclercq, 1994 cited by Wood, 2016). The uses of direct-fed useful microbial to chickens by administering with feed or drinking water is commonly practiced in commercial broiler production. The multispecies of microorganisms are more effective than monospecies (Timmerman et al., 2004). This is due to the interactive effects of anaerobes and facultative anaerobes. There are bacteria, photosynthetic bacteria, actinomycetes, and other types of organism. The microorganisms isolated from the digestive tract of chickens could increase the broiler productivity by 1.84-3.72% based on daily weight gain, feed efficiency, and mortality. The differences in the administration of microorganisms and timing of administration might affect the efficacy of microorganisms. The uses of microorganisms in the drinking water resulted in a lower average daily gain than using via the feed (Timmerman et al., 2005). The colonization patterns of chicks are instable and susceptible to pathogens. Initial colonization is important to the host because of a pioneer bacteria, which are the first to arrive in the gut, are capable of effectively blocking growth of other bacteria introduced later in the ecosystem. These pioneer bacteria also inhibit production of toxins by pathogenic bacteria (Ducluzeau, 1993). The microbial community of the gastrointestinal tract reflects the co evolution of microorganisms with their host and the diet adopted. Changes in the composition of the animal's microflora can have beneficial or detrimental effects on health, growth, and maturation of the animal host. Each region of the gastrointestinal tract developed its own unique bacterial community as the chicken matured (Lu et al.,2003). The objectives of this experiment were to determine the

effect of some microorganisms from the gastro-intestinal tracts of chicken and feed sterilization on the nutritive value of broiler diets.

MATERIALS AND METHODS

Isolation and Identification

The microorganisms were isolated from the broiler chickens' intestinal tracts content which was modified from Gonzalez-Pastor et al. (1994). The contents of duodenum, jejunum, ileum, caeca and large intestine were separated and removed under sterile conditions. Samples from each site were serially diluted in normal saline plated onto a NA (Nutrient Agar) medium for bacteria and actinomycetes and PDA (Potato Dextrose Agar) medium for fungi. They were incubated anaerobically and observed under compound microscope for morphological characteristics.

Enzyme production analysis

The isolated microorganisms were selected for study. They were screened for their ability to produce extracellular degradative enzymes such as amylase, protease, lipase, cellulase, hemicellulase and ligninase. Four replicates of each treatment were assayed and non-inoculated plates with substrates served as negative controls. The chemical indicators were added to assay enzyme activity and activity zones were measured.

Number of microbe determination

The isolated microorganisms which were capable to produces enzymes were selected for study as indicated in Table 1. The pour plate technique was used to determine the number of microbial in a plate. The range for total CFU/plate was between 135 to 160 colonies/plate for bacteria and 160 to 190 colonies/plate for actenomycetes, respectively. The fungal spore/plate was counted by Haemacytometer as follows; $3.1x10^8$ for *Aspergillus niger*, $1.7x10^8$ for *Rhizopus stolonifer*, $2.9x10^8$ for *Trichoderma* sp., and $2.1x10^8$ for *Muco*r sp.

Table 1. The efficiency of microorganism from Gi-tracts of broiler chickens on enzyme production.

Microorganism	Enzyme Production					
	Amylase	Protease	Lipase	Cellulase	Hemicellulase	Ligninase
Bacteria						
BC-NA-01	+		+	+	+	+
Actinomycetes						
BI-NA-03					+	+
BC-NA-02	+			+	+	+
BL-NA-01			+	+	+	+
Fungi						
Aspergillus niger (BD-PDA-01)	+	+				
Mucor sp. (BL-PDA-02)	+			+	+	+
Rhizopus stolonifer (BI-PDA-02)	+				+	
Trichoderma sp. (BL-PDA-02)	+		+	+	+	+

Diet sample preparation

Three rations of commercial broiler diet were used in this experiment. There were 22%, 20%, and 18% of crude protein for starter, grower, and finisher diet, respectively. The selected microorganisms were used at 5-7 days of age. Each plate of all selected fungi, bacteria and actenomycetes were mixed or non-mixed with 500 grams of broiler diets. They were sterilized or non-sterilized at 121°C with 15 psi. for 30 minutes. All diet samples were kept at room temperature for 30 days and their nutrients composition was determined by Proximate analysis.

Statistical analysis

The nutrient composition means served as the experimental unit with four replication, each for statistical analysis. Data were initially analyzed using the GLM procedure of SAS (SAS Institute, 1999) as a Completely Randomized Design (CRD) with factorial arrangements of microorganism supplementation level, sterilization level, and the 2-way interactions between microorganism supplementation and sterilization levels. Significant differences among treatments were determined at $P<0.05$ using Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSIONS

Effects of microorganism supplementation on nutrients composition

The nutrients composition was varied among treatment in three rations of broiler diets as indicated in Table 2. The microorganism supplementation was decrease crude fiber percentage in all three rations ($p<0.01$). The microorganisms that were used in this experiment were capable of producing enzyme cellulase, hemicellulase, and lignin which they can digest fiber in feedstuff. Furthermore, the microorganism supplementation in the diet was to improve the phosphorus percentage in the starter diet ($p<0.01$). Also it was to improve the calcium percentage both in the grower and finisher diet ($p<0.01$). However, the dry matter percentage was decreased in the starter, grower and finisher diets ($p<0.01$). The crude protein percentage was decreased only in the starter diet ($p<0.01$). Both the percentage of ash and metabolizable energy were not significantly different among treatment in the starter, grower, and finisher diets. The microorganism supplementation could not improve the importance nutrient composition as crude protein, ether extract, nitrogen free extract, and metabolizable energy because these selected microorganism in the experiment have less capability to produces enzymes which can digest protein, fat, carbohydrate, and energy in the diet.

Table 2. Effects of microorganism supplementation on nutrients composition of broiler diets.

Chemical Composition (%)	Starter		Grower		Finisher	
	With microorganism	Without microorganism	With microorganism	Without microorganism	With microorganism	Without microorganism
Moisture	10.75±0.07 [a]	9.42±0.08 [b]	11.27±0.10 [a]	9.66±0.11 [b]	11.50±0.0 [a]	9.91±0.07 [b]
Crude protein	19.42±0.16 [b]	20.25±0.1 [a]	17.51±0.39	18.54±0.46	16.69±0.63	16.98±0.25
Ether extract	5.60±0.22 [b]	6.33±0.16 [a]	7.19±0.18 [a]	7.80±0.20 [b]	6.43±0.26	6.94±0.19
Nitrogen free extract	55.00±0.47	54.33±0.29	56.03±0.42 [a]	55.49±0.58 [b]	57.34±0.75	57.18±0.32
Crude fiber	3.43±0.06 [b]	3.49±0.10 [a]	2.84±0.04 [b]	3.18±0.09 [ab]	2.89±0.07 [b]	3.51±0.04 [a]
Ash	5.75±0.16	6.14±0.10	5.14±0.10	5.32±0.11	5.15±0.06	5.47±0.11
Calcium	0.80±0.02 [b]	0.83±0.02 [a]	0.96±0.02 [a]	0.78±0.01 [b]	1.11±0.03 [a]	1.00±0.02 [b]
Phosphorus(%	0.46±0.02 [a]	0.31±0.02 [b]	0.19±0.02	0.42±0.02	0.30±0.03 [b]	0.32±0.02 [a]
Metabolizable energy (k.cal./kg.)	3,789.12 ±33.50	4,044.18 ±28.88	3,850.19 ±56.97	4,100.69 ±19.10	3,773.39 ±31.78	4,035.37 ±10.14

[a–b]Means within a row with no common superscripts differ highly significance ($P\leq0.01$).

Effects of sterilization on nutrients composition

The sterilized diet was varied in nutrient composition of broiler diets as indicated in Table 3. The crude protein and ether extract percentage was only increased in the starter diet ($p<0.01$). Also, the nitrogen free extract percentage was only increased in the finisher diet ($p<0.01$). The crude fiber and calcium were increased in both the grower and finisher diets ($p<0.01$). However, the dry matter percentage was lower in the starter, grower, and finisher diets ($p<0.01$) due to the moisture increased from the sterilization process. Metabolizable energy, ash, and phosphorus percentage were not significantly different among treatment in the starter, grower, and finisher diets. The crude protein percentage was also not significantly different among treatment in the grower and finisher diets.

Table 3. Effects of sterilization on nutrients composition of broiler diets.

Chemical Composition (%)	Starter		Grower		Finisher	
	Sterilize	Non-sterilize	Sterilize	Non-sterilize	Sterilize	Non-sterilize
Moisture	10.44±0.06 [a]	9.72±0.09 [b]	10.79±0.08 [a]	10.14±0.09 [b]	10.98±0.06 [a]	10.43±0.07 [b]
Crude protein	19.96±0.17 [a]	19.72±0.14 [b]	17.90±0.63	18.14±0.21	16.87±0.42	16.80±0.46
Ether extract	6.14±0.19 [a]	5.79±0.20 [b]	7.34±0.26 [b]	7.65±0.12 [a]	6.71±0.21	6.66±0.24
Nitrogen free extract	54.04±0.48 [b]	55.29±0.28 [a]	55.57±0.71	55.95±0.20	56.87±0.53 [a]	57.65±0.54 [b]
Crude fiber	3.45±0.08	3.47±0.08	3.14±0.06 [a]	2.88±0.08 [b]	3.24±0.03 [a]	3.16±0.07 [b]
Ash	5.92±0.16	5.97±0.09	5.23±0.07	5.23±0.12	5.31±0.09	5.30±0.07
Calcium	0.77±0.02 [b]	0.86±0.02 [a]	0.93±0.01 [a]	0.81±0.02 [b]	1.10±0.03 [a]	1.01±0.02 [b]
Phosphorus	0.39±0.02	0.38±0.02	0.30±0.02	0.30±0.02	0.32±0.03	0.30±0.01
Metabolizable energy(k.cal./kg.)	3,915.84 ±44.93	3,917.46 ±17.45	3,963.04 ±46.17	3,987.84 ±29.90	3,917.28 ±16.13	3,891.48 ±25.80

[a–b]Means within a row with no common superscripts differ highly significance ($P\leq0.01$).

Effects of microorganism supplementation and sterilize on nutrients composition

The interaction between of microorganism supplementation and sterilization on the nutrients composition was varied among treatments in the starter, grower, and finisher diets as indicated in Table 4-6. The microorganism supplementation with sterilization was higher in crude protein, ether extract, crude fiber, ash, and metabolizable energy than other treatments in the starter diet ($p<0.01$). However, crude protein, and metabolizable energy were not significantly different with the control treatment. Although, crude protein, ash and metabolizable energy were higher than other treatment but they were not significantly different with the control treatment in grower and finisher diets.

Table 4. Effects of microorganism supplementation and sterilize on nutrients composition of starter diets.

Chemical Composition (%)	With microorganism		Without microorganism	
	Sterilize	Non-sterilize	Sterilize	Non-sterilize
Moisture	9.63 [c]	10.24 [b]	11.26 [a]	9.21 [d]
Crude protein	20.26 [a]	19.19 [c]	19.66 [b]	20.25 [a]
Ether extract	6.78 [a]	5.71 [bc]	5.50 [c]	5.88 [b]
Nitrogen free extract	53.56 [c]	55.47 [a]	54.53 [b]	55.11 [ab]
Crude fiber	3.58 [a]	3.54 [a]	3.32 [b]	3.40 [b]
Ash	6.16 [a]	5.82 [b]	5.69 [b]	6.12 [a]
Calcium	0.66 [d]	0.71 [c]	0.89 [b]	1.01 [a]
Phosphorus	0.27 [d]	0.41 [b]	0.52 [a]	0.35 [c]
Metabolizable energy (k.cal./kg.)	4,053.49 [a]	3,800.04 [b]	3,778.20 [b]	2,034.88 [a]

[a–d]Means within a row with no common superscripts differ highly significance ($P\leq0.01$).

Table 5. Effects of microorganism supplementation and sterilize on nutrients composition of grower diets.

Chemical Composition (%)	With microorganism		Without microorganism	
	Sterilize	Non-sterilize	Sterilize	Non-sterilize
Moisture	9.76 [c]	10.73 [b]	11.81 [a]	9.55 [d]
Crude protein	18.33 [a]	17.54 [b]	17.48 [b]	18.74 [a]
Ether extract	7.77 [ab]	7.47 [b]	6.91 [c]	7.82 [a]
Nitrogen free extract	55.59 [b]	56.51 [a]	55.54 [b]	55.38 [b]
Crude fiber	3.21 [a]	2.60 [c]	3.08 [b]	3.15 [ab]
Ash	5.31 [a]	5.12 [b]	5.15 [b]	5.33 [a]
Calcium	0.94 [b]	1.00 [a]	0.92 [b]	0.62 [c]
Phosphorus	0.42 [a]	0.19 [b]	0.18 [b]	0.41 [a]
Metabolizable energy (k.cal./kg.)	4,819.75 [a]	3,864.05 [b]	3,836.32 [b]	4,111.63 [a]

[a–d]Means within a row with no common superscripts differ highly *significance* ($P\leq0.01$).

CONCLUSIONS

The supplementation of microorganism was decreased fiber and increased calcium, and phosphorus in broiler diets. The sterilization except the control treatment. method produced decreased dry matter in the diet. The supplementation of microorganism and sterilize method was higher in crude protein, ash, and metabolizable energy than the others

Table 6. Effects of microorganism supplementation and sterilize on nutrients composition of finisher diets.

Chemical Composition	With microorganism		Without microorganism	
	Sterilize	Non-sterilize	Sterilize	Non-sterilize
Moisture	10.02 [c]	11.06 [b]	11.94 [a]	9.8 [d]
Crude protein	17.05	16.69	16.68	16.90
Ether extract	6.95 [a]	6.36 [b]	6.49 [b]	6.93 [a]
Nitrogen free extract	57.10 [ab]	58.04 [a]	56.64 [b]	57.27 [ab]
Crude fiber	3.38 [b]	2.67 [d]	3.10 [c]	3.64 [a]
Ash	5.49 [a]	5.16 [b]	5.13 [b]	5.44 [a]
Calcium	1.09 [a]	1.11 [a]	1.10 [a]	0.91 [b]
Phosphorus	0.17 [b]	0.13 [b]	0.46 [a]	0.46 [a]
Metabolizable energy (k.cal./kg.)	4,318.05 [a]	3,750.26 [c]	3,796.51 [b]	4,312.69 [a]

[a–d]Means within a row with no common superscripts differ highly significance ($P\leq0.01$).

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