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Effects of natural bioactive mixture at varying levels on the growth and economic parameters of broilers (*Gallus gallus domesticus*)

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Abstract The results of the study ascertained that the rates of the natural bioactive mixture (combination of onion, lemon, and garlic juice) did not influence the weight of broilers and average daily gain at 7, 14, 21, and 28 days of rearing (P=>0.05). The weight of internal organs (liver, gizzard, gall bladder, spleen, esophagus, and lungs) and external organs (head, neck, back, wing, breast, feet, thigh, and drumstick) were not significantly different (P=>0.05). Among the parameters taken, it revealed significant results on the weight of the heart (P=<0.05), tail (P=<0.05), and return of feed cost (P=<0.01). The natural bioactive mixture at 30 ml L^{-1} improved the weight of the heart and tail of broilers at 28 days rearing period. However, it did not give more advantages to the overall improvement on the growth of broilers. Economically, the control treatment gave the highest return of feed cost, followed by 10 ml L^{-1} due to lesser inputs incurred during the production period. The findings of this study would provide additional evidence on the effects of the natural bioactive mixture on the growth of broilers. Hence, it is recommended to limit the inclusion of natural bioactive mixture as a supplement to broilers since there was a significant trend of increased heart and tail weights as the level of natural bioactive mixture increased.

Keywords: Bioactive substance, Chicken, Growth, Organs

Introduction

Today, herbs, spices, and medicinal plants have increased attention as possible growth promoters and immunity boosters. In many countries, routine use of antibiotics in poultry diets has been regulated. Thus, endeavors are made to develop new in-feed antibiotics substitutions to prevent, reduce, and treat infectious diseases in the poultry industry. The herbs and botanicals are increasingly being used in animal feed instead of antibiotics as a possible alternative means to prevent infectious diseases and improve the immune

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system responses (Wenk, 2003). Natural organic foods are generally believed to be healthier and less hazardous than food containing artificial flavors or additives. Supplementation of natural substances in the diets of broilers could reduce ammonia concentration while improving the feed conversion ratio (Raksasiri *et al*., 2018). Both onion and garlic are commonly used as phytogenic feed additives as an alternative to chemical growth promoters (Malematja *et al*., 2022) and (Munir, 2015). Onion plant parts contain compounds with proven antibacterial, antiviral, anti-parasitic, antifungal properties, antihypertensive, hypoglycemic, anti-thrombotic, antihyperlipidemic, and anti-inflammatory antioxidant activity (Walag *et al*., 2020).

The fresh onion stimulates blood circulation, improves immune response, and has antibacterial effects due to its pungent substances (Alçiçek *et al*., 2004). Garlic is a potential natural feed additive in poultry nutrition. It may be of great benefit and value, especially for broiler chicken due to its nutritional and medicinal properties. The presence of enzymes and other compounds could contribute to stimulating its growth (El-katcha *et al*., 2016). Another positive effect of garlic in the broiler is due to bioactive compounds such as allicin and diallyl sulfide. Due to its beneficial properties, garlic is a highly studied medicinal plant used to prevent diseases and a growth promoter in broiler chickens (Rehman *et al*., 2008). Calamansi or Philippine lime has beneficial properties commonly known as a source of vitamin C, antiseptic, antiphlogistic, carminative, deodorant, refrigerant, antimicrobial, hepatoprotective, expectorant, and antioxidant properties (Xin *et al*., 2022). The combination effects of lemon, garlic, and onion juice, improved the productive performance of chicken (Omer *et al*., 2019).

In this study, the researchers investigated the effect of natural bioactive mixture (calamansi, onion, and garlic juice) supplementation at different levels on broiler chickens' growth performance in Aurora, Zamboanga del Sur, Philippines.

Materials and methods

This experiment was intentionally conducted at the animal science experimental area of the Zamboanga del Sur Provincial Government College Aurora, Zamboanga del Sur, Philippines, from October 4 to November 1, 2020, covering 28 days. The study was conducted using a Complete Randomized Design (CRD) with four treatments and four replications. The treatments are as follows: Treatment 1- control (pure water); Treatment 2 - (10 ml L^{-1}); Treatment 3 - (20 ml L^{-1}); and Treatment 4 (30 ml L^{-1}). The experimental area

is well isolated and is free from disturbance. It has a good source of potable water needed for the birds for the study's whole duration. The perimeters of the site are eclosed with concrete walls. Sixteen elevated individual cages were constructed using bamboo sticks. The floor was built at a 1 x 1-meter using a plastic screen. Sides at 1 meter high were made of bamboo sticks at 2 cm spacing for proper ventilation. The roofs were made of galvalume sheets to maintain the ambient temperature of each cage. Disinfection of cages was done before the distribution of experimental birds. The researchers procured forty-eight heads of three-day-old broiler chicks from a commercial hatchery in Pagadian City, Zamboanga del Sur, Philippines. The broiler chicks were placed in the brooding cages to recover from stress upon arrival. The brooding stage of broiler chicks is a very important condition. Thus, the experimental birds were provided an artificial electric heat for 14 days to their respective cages. The chicks were weighed individually for their initial body weight and measured for their initial height, body length, and body circumference as benchmark data. There were three experimental birds in each cage for the whole rearing period. The researchers provided the experimental birds with commercial feeds throughout the trial period of 28 days. The three mash-type rations, such as chick booster, starter, and grower, were given at 0-10, 11-20, and 21-28 days of rearing.

The materials used to prepare the natural bioactive mixture were 12 kg lemon, 12 kg onion, 12 kg garlic, one basin, mortar and pestle, cloth, filter, chopping board, knife, and empty bottles. The juice of calamansi, onion, and garlic was extracted separately. First, calamansi juice was extracted and drained. Next, calamansi fruits were cut in the upper portion of the middle and squeezed manually direct to the bowl with a filter to separate the seed and juices, then poured in the medium basin and set aside. The onion was peeled and sliced into medium pieces, mashed manually by mortar and pestle, strained by a plain cloth, then squeezed to extract the onion juices. After extracting, the juices were put in the medium basin and set aside. The garlic bulb was packed into a single clove and smashed it manually by pressing it towards the wooden board. Smashed garlic was mashed for the second time using mortar and pestle, strained by plain cloth, and squeezed to extract the garlic juices. The different extracted juices were mixed in the big basin at a 1:1:1 ratio, then stirred and put in a sterilized bottle. The birds were administered a natural bioactive mixture by mixing it with the water according to each treatment's different levels. The cleaning of different cages was done regularly by the researchers. The dung under the cages was removed by sweeping. Federer and waterer were monitored and cleaned every time of administration of feeds and natural bio-active mixture supplementation. Used cartoons or papers were placed in each

cage as beddings to avoid chicks from foot injuries and minimize feed wastage. A canvass was installed to cover each cage's walls to protect the chicks from extreme temperatures.

Experimental birds were slaughtered manually using a sharpened knife after reaching the 28th day of rearing. The blood was drained separately and placed in a plastic ziplock. Dressing of birds was done, eviscerated, and cut by part separately in preparation for the birds' organs weight determination. The data gathered in this study were the weight, average daily gain, the weight of internal and external organs, feed conversion ratio, and return of feed cost. Data analysis was done using analysis of variance (ANOVA) in CRD using Minitab 17. The Fisher test was used to compare the mean difference. Relationships of some parameters were done using principal component analysis.

Results

Weight of broilers (g)

The weight of broilers at 0, 7, 14, and 21 days of rearing (DOR) is shown in Table 1. All experimental birds' weights were not far from each other since it revealed no significant results at 0 DOR. At 7 and 14 DOR, treatment 2 got the heaviest weights at 377.4 and 723.3 g., respectively. At 21 and 28 DOR, treatment 4 obtained the heaviest weights at 931.7 and 1,336.6 g., respectively. The data showed that the natural bioactive mixture (NBM) contributed a little increase in its growth compared to the control treatment. Statistical analysis did not reveal significant results on broilers' growth at 7, 17, 21, and 28 DOR.

Table 1. Weight of broilers at 0, 7, 14, 21, and 28 days of rearing

Treatments	Weight (g)				
	0 DOR	7 DOR	14 DOR	21 DOR	28 DOR
T_1 - Pure water (control)	125.0	344.7	653.3	832.3	1,219.2
T_2 – 10 ml L^{-1}	106.9	351.6	670.0	907.2	1,272.2
T_3 – 20 ml L^{-1}	123.3	377.4	723.3	925.2	1,273.3
T_4 – 30 ml L^{-1}	123.7	357.5	711.9	931.7	1,336.6
F-test	ns	ns	ns	ns	ns
C.V (%)	12.72	11.77	12.02	8.95	6.76

ns- non-significant

Average daily gain (g)

The average daily gain of broilers as influenced by NBM supplementation is presented in Table 2. Treatment 4 got the highest weight gain, as revealed by 43.3 g. Results suggested that the supplementation of NBM

at 30 ml L^{-1} had an advantage of 4.2 g. or 9.7% of the increase in broilers' average daily gain compared to control or pure water. Statistically, there was no significant difference in broilers' average daily gain at 28 DOR.

Table 2. Average daily gain of broilers as influenced by NBM at 28 DOR

Treatments	**Average daily gain (g)**
T_1 - Pure water (control)	39.1
T_2 - 10 ml L^{-1}	41.6
T_3 - 20 ml L^{-1}	41.1
T_4 - 30 ml L^{-1}	43.3
F-test	ns
C.V (%)	7.07

ns- non-significant

Table 3. Weight of external organs of broilers at 28 days of rearing (DOR)

Treatments	**Weight of external organs (g)**							
	Head	**Neck**	**Back**	**Wing**	**Breast**	**Feet**	**Thigh**	**Tail**
T_1 - Pure water (control)	36.50	52.00	109.75	104.00	288.50	48.75	116.00	8.25^{b}
T_2 – 10 ml L^{-1}	35.25	57.25	122.25	109.75	327.00	56.00	131.50	10.00^{ab}
T_3 – 20 ml L^{-1}	33.00	58.75	111.00	98.25	311.80	51.50	121.00	11.00^{a}
T_4 – 30 ml L^{-1}	33.25	62.75	116.00	102.75	331.25	54.00	125.00	10.75^{a}
F-test	ns	ns	ns	ns	ns	ns	ns	*
C.V (%)	11.55	11.96	11.95	10.77	9.93	9.63	9.40	16.33

ns- non-significant
*-significant at 5% level of Fisher pairwise comparisons

Weight of external organs (g)

The weight of the external organs of broilers at 28 DOR is shown in Table 3. Treatment 2 showed the heaviest weight of the head at 36.50 g, and back at 122.25 g, wings (109.75 g), feet (56 g), and thigh (131.50 g). Treatment 4 got the heaviest neck weight (62.75 g) and breast (331.25 g). Treatments 3 and 4 revealed the heaviest tail significantly, as revealed by 11 and 10.75 g., respectively, compared to control treatment at 8.35 g.

Table 4. Weight of internal organs of broilers at 28 DOR

Treat-ments	Weight of internal organs (g)							
	Liver	**Gizzard**	**Heart**	**Gall bladder**	**Spleen**	**Esophagus**	**Lung**	**Proven-ticulus**
T_1 - Pure water (control)	25.75	18.25	5.75[b]	2.50	1.75	2.13	6.75	8.50
T_2 - 10ml L^{-1}	29.50	18.25	7.00[ab]	2.50	2.40	2.40	7.25	10.50
T_3 - 20ml L^{-1}	25.75	17.25	6.25[b]	1.68	2.25	2.15	6.75	6.75
T_4 - 30ml L^{-1}	25.50	19.25	8.00[a]	1.98	1.90	2.00	7.00	10.25
F-test	ns	ns	*	ns	ns	ns	ns	ns
C.V (%)	13.71	16.56	18.34	40.45	28.11	29.77	16.20	46.44

ns- non-significant
*-significant at 5% level of Fisher pairwise comparisons

Weight of internal organs (g)

The weight of the internal organs of broilers at 28 DOR is presented in Table 4. Treatment 2 was consistent as the heaviest in terms of the internal organs like liver (29.50 g), gall bladder (2.50 g), spleen (2.40 g), esophagus (2.40 g), lungs (7.25 g), and proventriculus (10.50 g), although statistically insignificant. Treatment 4 showed the heaviest weight on the gizzard (19.25 g) and heart (8 g). Statistical analysis was not significant in the weight of gizzard and crop. On the weight of the heart, treatment 4 differed significantly from treatments 1 and 3. The weight of the heart in treatment 2 was not far from treatment 4.

Feed conversion ratio (FCR)

The feed conversion ratio of broilers in response to NBM supplementation is depicted in Table 5. Treatment 4 showed the best FCR in which the bird needs to consume only 1.78 kg of feeds to produce a kilogram of weight compared to the control, which needed to consume 1.98 kg of feeds. The lesser the FCR value expressed the more effectively the broilers gain weight. Statistical analysis in FCR was not significant, although a little contribution of NBM supplementation was evident.

Table 5. Feed conversion ratio of broilers as influenced by NBM supplementation

Treatments	Feed conversion ratio
T_1 - Pure water (control)	1.98
T_2 – 10 ml L^{-1}	1.85
T_3 – 20 ml L^{-1}	1.89
T_4 – 30 ml L^{-1})	1.78
F-test	ns
C.V (%)	7.55

ns=non significant

Return of feed cost (RFC)

The return of feed cost (RFC) of broilers supplemented with NBM is presented in Table 6. The RFC of broiler in treatment 1 was significantly higher than the rest of treatments, as revealed by 84.98%. Treatment 2 was 62.40 which significantly differed from treatment 3 (42.76) and treatment 4 (34.13). The highest RFC in treatment 1 was due to the lesser inputs incurred during the production. Among the NBM levels, treatment 2 obtained the highest RFC. Economically, the results showed that treatment 1 was the cheapest in all treatments observed.

Table 6. Return of feed cost of broilers in response to NBM supplementation

Treatments	Return of feed cost
T_1 - Pure water (control)	84.98^{a}
T_2 – 10 ml L^{-1}	62.40^{b}
T_3 – 20 ml L^{-1}	42.76^{c}
T_4 – 30 ml L^{-1}	34.13^{c}
F-test	**
C.V (%)	40.10

**=highly significant at 5% level of significance of Fisher Test

Scree plots

The scree plots on the relationships of selected parameters are shown in Figure 1. It showed the principal component (PC) numbers and eigenvalues that corresponded to the total variability of the data. As reflected, PCs 1 and 2 are considered in Fig. 1a, PCs 1-3 in Fig. 1b, and PCs 1-4 in Fig. 1c, which accounted for the most of the total data variability as reflected in the eigenvalues of more than 1.

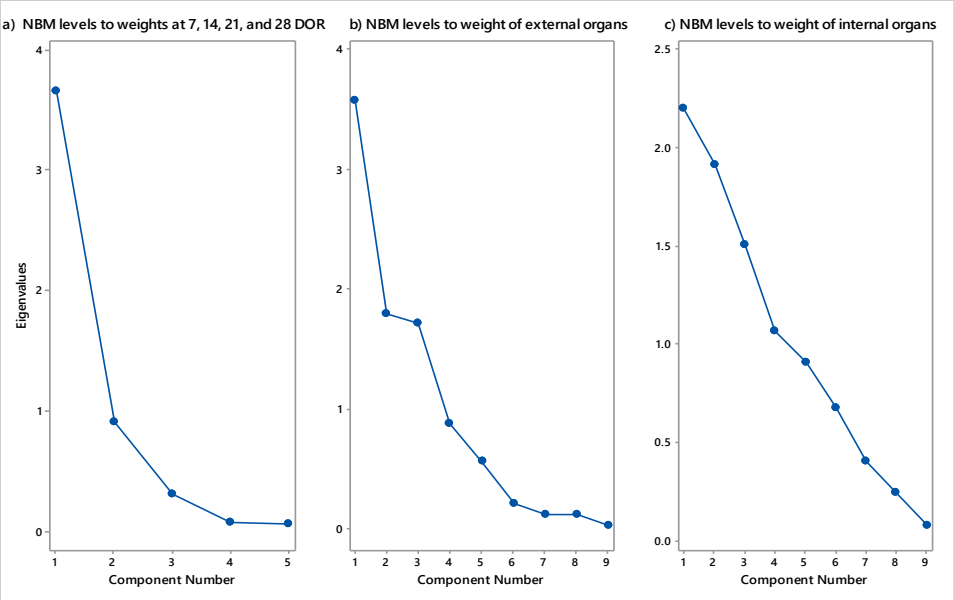

Figure 1. Scree plots on the relationships of selected parameters

Principal component analyses of selected parameters

The principal component analysis of NBM levels to weights of broilers at 7, 14, 21, and 28 DOR is shown in Table 7. The weights at 7 DOR (0.388), 14 DOR (0.442), 21 DOR (0.460), 28 DOR (0.444), and ADG (0.431) had the highest positive loadings in component 1 showing that the ADG increased as the weights at 7, 14, 21, and 28 DOR increased. In PC 2, the NBM levels had the highest positive loading, while the weight at 7 DOR had the highest positive loading. Thus, it revealed an increment of broilers' weight with the increasing level of NBM supplementation.

Table 7. Principal component analysis of NBM to the weight of broilers at 7, 14, 21, and 28 DOR

Variables	PC1	PC2	PC3	PC4
NBM levels	0.243	-0.792	-0.548	0.099
Weight at 7 DOR	0.388	0.476	-0.422	0.619
Weight at 14 DOR	0.442	0.278	-0.196	-0.368
Weight at 21 DOR	0.460	0.080	-0.087	-0.610
Weight at 28 DOR	0.444	-0.144	0.444	0.292
ADG	0.431	-0.204	0.527	0.115
Cumulative proportion	0.739	0.897	0.976	0.989
Percentage	73.9	89.7	97.6	98.9

The principal component analysis of NBM levels to the external organs of broilers is presented in Table 8. The weights of the neck (0.382), back (0.354), breast (0.452), feet (0.400), and thigh (0.444) had the highest positive loadings in component 1. It showed an increase in the weight of the breast as the weights of the neck, back, feet, and thigh increased. In PC2, the NBM levels (-0.408) and neck (-0.447) had the highest negative loadings, while the wing's weight had the highest negative loading. It revealed that when the level of NBM is decreased, the weight of the neck increased while the weight of the wing increased. In the PC3, the NBM level (-0.439) and weight of the tail (-0.568) had the highest negative loadings, while the weight of the head (0.608) had the highest positive loading. The result implied an increase in the weight of the head while lowering the weight of the tail as the level of NBM is reduced.

Table 8. Principal component analysis of NBM to broilers' external organs

Variables	**PC1**	**PC2**	**PC3**	**PC4**
NBM levels	0.276	-0.408	-0.439	0.107
Weight of head	0.025	0.003	0.608	0.589
Weight of neck	0.382	-0.447	0.160	-0.003
Weight of back	0.354	0.287	0.074	-0.596
Weight of wing	0.185	0.649	-0.115	0.168
Weight of breast	0.452	-0.217	0.161	-0.157
Weight feet	0.400	0.050	0.207	0.144
Weight of thigh	0.444	0.274	0.043	0.097
Weight of tail	0.238	0.043	-0.568	0.450
Comulative proportion	0.396	-0.568	0.786	0.884
Percentage	39.6	56.8	78.6	88.4

Table 9. Principal component analysis of NBM to broilers' internal organs

Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
NBM levels	0.144	-0.373	0.551	0.290	-0.207
Weight of liver	0.566	0.013	-0.318	-0.259	-0.063
Weight of gizzard	-0.288	-0.499	-0.295	0.059	0.141
Weight of heart	0.220	-0.322	0.505	-0.353	-0.249
Weight of gall bladder	-0.253	0.395	0.055	-0.361	-0.322
Weight of spleen	0.556	-0.007	-0.008	-0.311	0.401
Weight of esophagus	-0.259	-0.227	-0.132	-0.608	-0.333
Weight of lungs	0.344	0.124	-0.284	0.350	-0.697
Weight of proventriculus	-0.011	-0.534	-0.390	0.026	-0.103
Comulative proportion	0.245	0.457	0.624	0.743	0.844
Percentage	24.5	45.7	62.4	74.3	84.4

The principal component analysis of NBM levels to different weights of internal organs is presented in Table 9. As presented, liver weights (0.566) and spleen had the highest positive loadings. The result suggested an increase in the weight of the spleen when the weight of the liver is increased. In PC2, the NBM levels (-0.373), the weight of gizzard (-0.499), the weight of heart (-0.322), the weight of proventriculus (-0.534) had the highest negative loadings, while the weight of the gall bladder had the highest positive loadings. It showed a decrease in the gizzard's weight, heart's weight, and proventriculus's weight when the level of NBM is reduced. In PC3, the NBM levels (0.551) and weight of the heart (0.505) had the highest positive loadings, while the liver (-0.318) and weight of proventriculus (-0.390) had the highest negative loadings. The data revealed that the weight of the liver and the proventriculus decreased while the weight of the heart increased when the levels of NBM increased.

Discussion

The NBM with the onions and calamansi as the ingredients have been studied to improve cholesterol and low-density lipoprotein (Goodarzi *et al*., 2013). However, too much consumption of onion and garlic in the diet may have possible bad effects on the users (Kendler, 1987). In the case of broilers, when supplemented with NBM, the weight of the tail is increased due to fats and cholesterol build-up. Nevertheless, the weights of external organs of broilers as investigated like head, neck, back, wings, breast, feet, and thigh did not differ significantly.

Natural bioactive substance supplementation composed of calamansi, onion and garlic extract has been identified to have many substances, improving the broilers' immune defense system. Onion and garlic were studied and discovered to obtain the two types of lipoprotein, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol (An *et al*., 2015). Low-density lipoprotein cholesterol or bad cholesterol transports all fat molecules in the intracellular muscle around the body. Its function is to deliver all types of fats around the body. The high-density lipoprotein like a vacuum cleaner for cholesterol in the body (Rahimi *et al*., 2011). Hypocholesterolemic effects with the onion diet contribute to decrease cholesterol secretion from the liver, leading to increase uptake of high-density lipoprotein cholesterol into the heart. It had been observed that the broilers fed diet with 3% onion was significantly produced higher lipoprotein and lower blood triglycerol levels (Rohatgi *et al*., 2014). A study was also shown that garlic powder can be significantly increased the high-density lipoprotein levels and lower serum and liver cholesterol. Plasma cholesterol and plasma high-density lipoprotein in animals resulted from the highest dietary level of garlic (Horton *et al*., 1991).

The most broilers encountered heart problems due to the high consumption of amino acids in their diet. On the other hand, limiting the inclusion of amino acids in broilers may lower growth performance (Golshahi *et al*., 2013). Furthermore, the slaughter age of broilers is shortened due to growth hormones. Thus, fast-growing broilers may encounter heart problems that are detrimental to the broiler industry (Olkowski, 2007). With the observation of results and based on some references, the 30 ml of NBM per liter of water improved the two lipoproteins, resulting in a heavier heart and tail compared to the rest of the treatments. The natural bioactive mixture resulted in beneficial effects for broilers, but too much consumption may cause undesirable effects. Hence, it is recommended to limit the inclusion of natural bioactive mixture in the broilers' diet not to exceed 20 ml L^{-1}.

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