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DIETARY SUPPLEMENTATION OF SECONDARY PLANT COMPOUNDS AND ZINC BACITRACIN ON GROWTH PERFORMANCE AND LIPID PROFILE OF BROILERS

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Broiler industry is currently looking for alternatives to increase the efficiency of feed consumption while eliminating the use of antibiotic growth promoters (AGP). This study was conducted to investigate the effect of supplementing broiler chicken diets with secondary plant compounds (SPC) and zinc bacitracin on growth performance and serum lipid profile. Three hundred, eight day-old Cobb500 broiler chicks were randomly allocated to five experimental diets with six replicates each in a completely randomized design. The supplements were mixed in the commercial diet at two different levels (SPC; 10% and 20% Activo® and AGP; 20% and 40% Zinc bacitracin (ZB)). Broilers fed on the commercial broiler diets without supplements were considered as the control. Body weights and feed consumption were recorded at weekly intervals and body weight gain and feed conversion ratio (FCR) were calculated. On 42nd day, birds were slaughtered and dressed weight, internal organs weight, and serum lipid profile were measured. Data were analyzed using one way Analysis of Variance in SAS. Dietary intake of 20% Activo® resulted significant increase ($p<0.05$) in body weight gain and decrease in FCR compared to the control group. However, weight gains and FCR were similar in ($p>0.05$) birds fed with different percentages of ZB and Activo®. Carcass and internal organ weights were not significantly different ($p>0.05$) among the treatments. Inclusion of supplements significantly reduced ($p<0.05$) the serum total cholesterol and low density lipoprotein compared to the control. Therefore, secondary plant compounds used in the study can be utilized at a level of 20% as an alternative to antibiotic growth promoters to improve broiler performance to reduce the serum cholesterol.

Keywords: Antibiotic growth promoter, Broilers, Growth performance, Secondary plant compound, Serum lipid profile

As the broiler industry became commercially profitable, the industry wanted to make it more effective and efficient hence they tried to utilize the best available technology to uplift its standards. The poultry industry has developed in several areas such as nutrition and genetics management to maximize the efficiency of growth performance and meat yield (Gunal *et al*., 2006). In the nutrition aspects of poultry, huge development had occurred during the past few decades especially the use of antibiotics to increase the performance has been popularized. However, nowadays, the poultry industry has focused more attention towards addressing the public concern for environmental and food security (Anon, 2015; Cervantes, 2012; Chowdhury, 2009). Antibiotic feed additives as growth promoters (AGP) have been under scrutiny and many have been removed from the market (Ratcliff, 2000). With the newfound interest towards the negative impacts of AGP the requirement for the alternatives to AGP (Kocher and Choct, 2008) has become an important aspect in modern research.

So as an alternative to AGP, secondary plant compounds (SPC) can play a decisive role. Secondary plant compounds such as essential oils can be used to replace AGP. Essential oils derived from herbs and have been long recognized for their anti-microbial

Table 1: Ingredient composition of basal ration (as-fed basis)

Ingredient	**Composition (%)**		
	Booster	**Starter**	**Finisher**
Broken rice	21.25	47.00	55.40
Maize	31.00	0.00	0.00
Soybean meal	6.50	21.00	14.70
DDGS	5.50	7.00	12.00
Rice polish	0.00	3.00	0.00
Meat bone meal	1.25	5.50	6.50
Bakery meal	0.00	4.50	0.00
Wheat shorts	0.00	3.50	0.00
Vegetable oil	2.00	2.75	4.80
Fish meal	3.00	0.00	0.00
Corn gluten meal	0.20	3.00	4.00
Dicalcium phosphate	0.50	0.25	0.30
Limestone powder	0.35	0.62	0.35
L-Lysine (98%)	0.28	0.31	0.44
DL-Methionine (98.5%)	0.20	0.22	0.24
NutriMin CPM116	0.12	0.20	0.20
L-Threonine (98.5%)	0.18	0.12	0.10
Sodium bicarbonate	0.10	0.15	0.30
Toxin binder	0.01	0.05	0.05
Garlic powder (25%)	0.01	0.15	0.01
VC 90%	0.02	0.01	0.00
Betain	0.01	0.02	0.02
Antioxidant	0.01	0.01	0.02
phytase5000	0.04	0.01	0.01
1% Maduramicin	0.00	0.04	0.05
4% Salinomycin	0.05	0.00	0.00
Vitamin MPV118	0.05	0.04	0.03
Yiduozyme8601	0.04	0.05	0.03
Mould inhibitor	0.02	0.04	0.04
Probiotics-100	0.15	0.01	0.01
Salt	0.25	0.20	0.30
Choline Chloride (60%)	0.10	0.08	0.05

activity (Lee *et al*., 2004). They have gained much attention for their potential as alternatives to antibiotics. Essential oils have been shown to improve gut health by modulating the intestinal microbiota, stimulating enzyme activity and animal performance. Supplementation of essential oils to poultry has shown to stimulate the production of endogenous enzymes. Feed digestion is thus enhanced and modulate positively the microbiota, resulting in a reduced risk of enteric disease (Jang *et al*., 2004).

With the restriction of antibiotic usage, huge demand has been created for an alternative, which could replace the use of antibiotics. Secondary plant products are the ideal candidate to fill this void. Therefore, the aim of this study was to compare the growth performance parameters of broilers fed diets supplemented with a blend of essential oil commercially named Activo® and

commercial antibiotic growth promoters (Zinc Bacitracin) in Sri Lankan context.

MATERIALS AND METHODS

The experiment was conducted at the trial farm of New Hope Lanka Ltd., Ekala, Ja-Ela, Sri Lanka. Laboratory analyses were conducted at the laboratory of New Hope Lanka Ltd., and Animal and Food Science laboratory, Faculty of Agriculture, Rajarata University of Sri Lanka.

Experimental diets

Two concentrations of AGP and SPC were added to the basal feed mixture as feed additives and treatments were as follows;

Treatment 1: standard feed without additives (control)

Treatment 2: standard feed with 20% antibiotic growth promoters (Zinc Bacitracin)

Treatment 3: standard feed with 40% antibiotic growth promoters (Zinc Bacitracin)

Treatment 4: standard feed with 10% secondary plant compounds (Activo)

Treatment 5: standard feed with 20% secondary plant compounds (Activo)

Feed mixing

The floor was cleaned to avoid contamination of any foreign materials. Macro ingredients and micro ingredients were weighed (**Error! Reference source not found.**) and mixed by a horizontal double ribbon type feed mixer. While macro and micro ingredients were mixing, vegetable oil was incorporated into the mixture. Treatment additives were mixed using a shovel. For the booster, starter and finisher period, 120 kg, 500 kg and 675 kg of feed were prepared, respectively. Prepared diets were allocated to each pen and stored in labeled bags until feeding.

Animals and experiment design

Day old chicks of Cobb 500 were purchased from a commercial hatchery, Chemical Industries Colombo (CIC) Ltd., Sri Lanka. A total of 300 broiler chicks were randomly assigned into five treatments with six replicates per each. Each treatment was comprised of 60 birds, where 10 birds were allocated per replicate. The experiment was conducted as a Complete Randomized Design (CRD).

Other management practices

Ten days prior to the commencement of the experiment, the pens were washed using disinfectant. The floor was treated with Limestone. Feeders and waterers were washed and disinfected. Wood shavings were used as the litter material. Before introducing chicks, pens were covered by black polythene to protect from wind, rain, and predators. Brooding temperature was provided by 100W electric bulbs. Day old chicks were introduced to pre-heated brooder pens and brooded up to 8 days. Just after chicks were introduced to the pens, all chicks were provided with Vitamin E (Selvite-E) and glucose solution with drinking water to reduce the stress. All birds were vaccinated against infectious bursal disease (IBD) at age of 7 and 14 days. On day 8, birds were moved from brooders to the experimental pens.

Chemical analysis of feed samples

Feed samples (100 g) were collected randomly after the mixing of feeds. Those samples were dried and ground to a fine powder, then stored in sample bags until analysis. Experimental diets were analyzed to determine moisture, crude protein (CP), crude fiber (CF), crude fat (EE), crude ash (CA), calcium (Ca), and phosphorus (P). Proximate analysis of feed samples was done according to the standard methods of the Association of Official Analytical Chemist (AOAC, 1995).

To evaluate the moisture 3.0 g of feed samples were dried at 103℃ for 4 hours in the dry oven (model-GZF6020, China). Soxhlet extraction system (DKZW-4, China) was used to measure crude fat contents of the sample by allowing eight hours ether extraction. The crude protein content of the feed samples was measured using the Kjeldahl apparatus (VAPO45, Germany). Ash content was measured after ignited the 1 g of sample in a furnace (SX-4-10, China) set at 550℃ for 4 hours. Calcium and phosphorus were analyzed using spectrophotometer (Model- 1188Q08EA, China).

Slaughtering of birds

At the end of the study period (42 days), birds were randomly selected from each pen and weighed. Birds were killed by dislocating neck and hanged until bleeding was completed. Feathers were removed. Then a cut was made at the end of the abdomen and abdominal cavity was opened. The digestive tract, respiratory tract, heart, and liver were removed and gallbladder was peeled away. Gizzard was cleaned and the inner layer was removed. Breast, thighs, and drumsticks with bone were then cut and skin was peeled using a scalpel blade and weighed.

Serum lipid profile

Blood serum parameters were measured when birds slaughtered at day 42. During bleeding, blood samples were collected from randomly selected fasting birds from each replicate to sterilized tubes (without anticoagulant). Immediately, serum was separated by centrifugation at 1500 rpm for 20 min. (model-C0060, USA). Then, samples were stored in -20^{0}C until analysis. The enzymatic colorimetric test was used to determine the total serum cholesterol, high-density lipoprotein (HDL) and triglyceride according to the manufactures (BIOLABO, France) guidelines. Low-density lipoprotein (LDL) content was calculated by subtracting the HDL cholesterol content from total cholesterol content.

Data collection statistical analysis

Given feed quantity and remaining quantity were measured daily and then feed intake was calculated throughout the experimental period. Body weight was measured weekly and weight gain was calculated. Feed conversion ratio (FCR) was calculated. Live weight at slaughtering and carcass weights were recorded and dressing percentage was calculated. The weight of the internal organs; liver, gizzard, small intestine, caeca, pancreas, and heart were measured and expressed as percentages of the live slaughter weight.

Weight gain, feed intake, FCR, dressing percentage of birds and serum lipid profile were analyzed using the MIXED procedure of Statistical Software for Data Analysis (SAS), Ver 9.0 (SAS, 2002). Mean separation was done by Turkey's Standardized Range Test (TSRT). Statistical significance was declared at $P<0.05$.

RESULTS AND DISCUSSION

Proximate compositions of treatment diets

Proximate compositions of the treatment diets were presented in Table 2. The moisture, CP, EE and ash contents of the booster, starter and finisher diets more or less similar to the observed values by Ahmad (2018) and recommended values by National Research Council (1994).

Growth performances

Table 2: Proximate compositions of the treatment diets

Diet	Treatment	Moist[1] (%)	CP[2] (%)	CA[3] (%)	EE[4] (%)	CF[5] (%)	Ca[6] (%)	P[7] (%)
Booster	Control	11.99	20.8	5.45	3.97	2.05	1.02	0.65
	ZB 20%	12.72	21.6	4.96	4.76	2.14	0.99	0.56
	ZB 40%	12.62	22.4	5.24	5.82	2.06	1.12	0.59
	Act10%	13.13	21.4	5.45	5.75	2.33	1.15	0.62
	Act20%	13.05	21.7	5.12	5.95	2.32	1.20	0.72
Starter	Control	10.87	22.8	7.41	4.84	2.79	1.20	0.66
	ZB 20%	11.04	22.5	7.52	3.57	2.84	1.01	0.59
	ZB 40%	11.02	22.4	8.18	3.89	2.74	1.05	0.64
	Act10%	10.80	21.3	7.62	4.38	2.64	1.13	0.67
	Act 20%	11.02	21.1	8.90	4.53	2.83	1.16	0.68
Finisher	Control	10.50	20.0	5.41	6.96	2.21	0.98	0.71
	ZB 20%	10.92	20.1	4.98	7.13	2.03	0.95	0.69
	ZB 40%	10.23	19.6	4.23	7.83	2.21	1.05	0.58
	Activo10%	10.89	19.9	5.34	6.88	2.24	1.14	0.65
	Activo20%	10.46	20.1	5.32	7.19	2.23	0.96	0.62

[1] Moisture, [2]Crude protein, [3]Crude ash, [4]Crude fat, [5]Crude fiber, [6]Calsium, [7]Phosphorus

Table 3: Average body weights of broilers fed with different levels of Zinc bacitracin and Activo

Age	Body weight of different treatments (g/bird) *					SE
	Control	ZB20%	ZB40%	Act10%	Act20%	
Day 8 – 14	419[b]	439[a]	431[ab]	434[ab]	443[a]	5
Day 15 – 21	848	860	855	863	860	6
Day 22 – 28	1337[b]	1368[a]	1355[ab]	1357[ab]	1365[ab]	10
Day 29 – 35	1812[b]	1860[a]	1860[a]	1863[a]	1867[a]	12
Day 36 – 42	2367[b]	2417[ab]	2467[ab]	2417[ab]	2517[a]	52

Data are presented as means ± Standard Error (SE)

[a,b] means within the same raw with different superscripts are significantly different (P<0.05).

* ZB 20% = 20% Zinc bacitracin, ZB 40% = 40 % Zinc bacitracin, Act 10% = 10% Activo®, Act 20% = 20% Activo®.

Table 4: Growth performance of broilers fed with different levels of Zinc bacitracin and Activo®

Parameters	Treatments*					SE
	Control	ZB20%	ZB40%	Act10%	Act20%	
Weight gain (g/bird)						
0 - 21 days	634	642	640	647	639	5
22 - 42 days	1518[b]	1557[ab]	1612[ab]	1553[ab]	1657[a]	52
0 - 42 days	2152[b]	2199[ab]	2252[ab]	2201[ab]	2296[a]	51
Feed intake (g/bird)						
0 - 21 days	1032	1044	1018	1029	1032	9
22 - 42 days	2569	2555	2522	2527	2523	19
0 - 42 days	3601[a]	3581[ab]	3507[b]	3523[ab]	3555[ab]	28
Feed conversion ratio						
0 - 21 days	1.63	1.63	1.61	1.59	1.62	0.02
22 - 42 days	1.70[a]	1.64[ab]	1.58[ab]	1.63[ab]	1.53[b]	0.05
0 - 42 days	1.68[a]	1.64[ab]	1.58[ab]	1.62[ab]	1.55[b]	0.03

Data are presented as means ± Standard Error (SE)

[a,b] means within the same raw with different superscripts are significantly different (P<0.05).

* ZB 20% = 20% Zinc bacitracin, ZB 40% = 40 % Zinc bacitracin, Act 10% = 10% Activo®, Act 20% = 20% Activo®.

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Effects of treatments on average body weight of broilers are presented in **Error! Reference source not found.**3. There were significant differences (P<0.05) in weekly body weights of birds fed with different treatment diets except during the day 15 - 21 (**Error! Reference source not found.**3). From day 8 - 14, body weights of birds supplemented with Activo 20% and ZB 20% were significantly higher (P<0.05) compared to the broilers fed with control diet. However, body weights of birds supplemented with AGP and SPC were similar during the period (P>0.05). Body weight of birds fed with 20% ZB was higher (P<0.05) than the birds fed with control diet from day 22 - 28. The body weights of birds fed with AGP and SPC were significantly higher (P<0.05) than the control during day 29 - 35.

Body weight gains of birds fed with different treatment diets were similar (P>0.05) during the starter period (Table 4). There was a significant difference in (P<0.05) body weight gain of birds fed with different treatment diets during the finisher period and total study period. Body weights of birds supplemented with Activo 20% was

higher (P<0.05) than the birds fed with control diet. According to the Alp *et al.* (1999) and Lea and Rainer (2015), addition of Acid Lac Dry+zinc bacitracin and 110 ppm bacitracin to the diet, respectively resulted in greater weights in broilers. However, the addition of AGP showed only a numerical increase in weight gain compared to the control. Further, according to the Lea and Rainer (2015) body weight gain was increased in the Tylosin 40 ppm, Activo 100 g/t, and Activo+Enramycin 8 ppm supplemented diets than the control with no supplements. Similar findings were observed in this study by adding Activo 20% to the broiler diets which resulted increased body weight of broilers.

Feed intake of birds fed with different treatment diets were similar (P>0.05) during the starter period and finisher periods (Error! Reference source not found.4). However, there was a significant difference (P<0.05) in feed intake of birds fed with different diets during the whole study period. Feed intake of broilers fed with control diet was significantly higher (P<0.05) compared to the 40% ZB supplemented the diet and numerically higher than the other treatment diets. These results are agreed with the findings of the Lea and Rainer (2015), who showed that the highest feed intake in the no additives diet than the AGP supplemented diets.

Feed conversion ratio of birds fed with different treatment diets were similar during the starter period (P>0.05) and significantly different (P<0.05) during the finisher and the whole study period (Table 4). During the finisher and total study period, FCR was better (P<0.05) in birds fed with the diet containing Activo 20% than the control diet. This might be due to the digestion stimulatory and the gastro-protective effect as reported by Abdulla *et al.*, (2010) and Enburg *et al.*, (2000). Results of this study was agreed with Denli (2004), who found that birds offered the diets containing "thyme" one of the essential oils at 60 mg/kg in feed exhibited increased (P<0.05) body weight gains and improved the feed efficiency as compared with the control group (P<0.05).

Carcass characteristics of broilers

Effect of different treatment diets on the carcass characteristics of broiler chicken are presented in Table 5. The results of the experiment indicated that 20% Activo supplementation improved (P<0.05) the live weight of birds compared to control and there was no any difference in body weight among the AGP and SPC supplemented birds. Further, there was no any significant difference (P>0.05) of the carcass yield and dressing percentages of birds, among the treatment diets. These results agreed with Alp *et al.*, (1999) who concluded that dressing percentage was unaffected by organic acids and zinc bacitracin supplementation regardless of they were fed alone or in combination. Moreover, the percentage of the drumstick, thigh, heart, gizzard, liver, pancreas, small intestine were not significantly different (P>0.05) among the birds fed with different treatment diets.

Effect of treatments on blood serum parameters of broilers

The serum total cholesterol and LDL content of birds fed with different treatment diets were significantly different (P<0.05) and birds fed with control diet reported high serum cholesterol and LDL compared to the birds supplemented with AGP and SPC (**Error! Reference source not found.**6). However, total cholesterol levels and LDL were similar in birds fed with diets containing AGP and SPC. These results were agreed with the Elagib *et al.*, (2017) who reported, decreased levels of LDL concentrations with the inclusion of various levels of (0.2, 0.4 and 0.8 g /kg) *C. cyminum* essential oil into the broiler diets, at day 28.

However, Elagib *et al.*, (2017), observed significantly reduced serum cholesterol and LDL in spices supplemented birds compared to the "doxystin" (Antimicrobial drug) treated birds and the control groups. Also, Gaucher *et al.*, (2015) revealed that total cholesterol of the serum, thigh and breast meat were reduced with the cinnamon oil supplementation.

There was no significant difference (P<0.05) in serum triglycerides and HDL contents among the birds fed with different treatment diets (Table 6). However, Aruna *et al.*, (2005) found that one of the secondary plant compounds, *C. cyminum* significantly

reduced the plasma triglycerides levels of broilers.

Mortality rate

There was no significant difference (P<0.05) in mortality rate (0 - 3%) among the birds fed with different treatment diets. Compared to the other treatment diets, birds supplemented with 20% Activo has represented the lowest mortality rate (0%). These findings were agreed with the Freitas *et al.*, (2012) and the mortality rate of birds fed with essential oil is lower than that of the birds fed with AGP.

Table 5: Carcass characteristics of broilers fed with different levels of Zinc bacitracin and Activo®

Parameters	Treatments*					SE
	Control	ZB20%	ZB40%	Act10%	Act20%	
Live weight (g)	2350[b]	2450[ab]	2450[ab]	2417[ab]	2517[a]	68
Carcass weight (g)	1653	1707	1720	1705	1760	50
Dressing percentage (%)	70	70	70	71	70	0.65
Drumstick (%)	5	5	5	5	5	0.20
Thigh (%)	4	4	4	4	5	0.19
Internal organs (% live weight)						
Heart	0.44	0.44	0.47	0.51	0.46	0.03
Gizzard	0.93	0.97	0.95	0.90	0.90	0.03
Liver	0.22	0.23	0.22	0.20	0.20	0.03
Pancreas	0.18	0.20	0.20	0.18	0.19	0.02
Small intestine	2.55	2.63	2.55	2.43	2.53	0.11

Data are presented as means ± Standard Error (SE)

[a,b] means within the same raw with different superscripts are significantly different (P<0.05).

* ZB 20% = 20% Zinc bacitracin, ZB 40% = 40 % Zinc bacitracin, Act 10% = 10% Activo®, Act 20% = 20% Activo®.

Table 6: Blood serum parameters of broilers fed with different levels of Zinc bacitracin and Activo®

Parameters** (mg/dL)	Treatment*					SE
	Control	ZB20%	ZB40%	Act10%	Act20%	
TCH	237[a]	160[b]	171[b]	205[b]	163[b]	25
TAG	29	39	36	33	30	4
HDL	85	88	83	89	88	3
LDL	151[a]	72[b]	88[b]	115[b]	75[b]	26

Data are presented as means ± Standard Error (SE)

[a,b,] means within the same column with different superscripts are significantly different (P<0.05).

* ZB 20% = 20% Zinc bacitracin, ZB 40% = 40 % Zinc bacitracin, Act 10% = 10% Activo®, Act 20% = 20% Activo®.

**TCH = Total cholesterol, TAG = Triglycerides, HDL = High density lipoprotein, LDL = Low density lipoprotein.

CONCLUSIONS

The results indicate that the secondary plant compounds used in the study can be utilized at a level of 20% as an alternative to antibiotic growth promoters to improve broiler performance and to reduce the serum cholesterol

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