



Effect of dietary supplementation of humic acids on performance of broilers

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ABSTRACT

An experiment was conducted to study the efficacy of humic acid (HA) based product as a substitute for antibiotic in broilers. Two basal diets, viz. moderate (BIS 1992) and low nutrient density diets (3.5% lesser ME and CP than BIS 1992 specification) were formulated. Low and moderate nutrient density basal diets without antibiotic were served negative controls and with added antibiotic became positive controls. Further, moderate and low nutrient density diets were supplemented with 0.5, 0.75 or 1 g HA based product per kg of feed to result in 6 test diets. Each of such diets prepared for starter (0–14 days), grower (15–28 days) and finisher (29–42 days) phases were offered to duplicate groups of 14 chicks each. The results revealed significantly higher body weight gains in moderate density diet with 0.1% HA during starter phase, more feed consumption in low nutrient density diet with 0.075% HA during finisher stage and better feed efficiency in moderate density diet with 0.075% HA. However, the cumulative body weight gain, feed consumption and feed efficiency including net returns were comparable among different treatments during 42-day trial. Metabolizability of dry matter and crude protein was significantly high in groups fed moderate nutrient density diet than low nutrient density diets however, no definite trend was observed with HA supplementation. At the termination of 42-day-trial, no significant differences were observed in dressing percentage, breast-meat yield, abdominal fat pad, relative weights of liver, heart, gizzard, spleen and bursa among different treatments. On 10-day post-vaccination, the ND titer values were statistically similar among different treatments while improved IBD titer values of birds were observed in HA supplanted groups. It was concluded that the addition of HA up to 0.1% particularly in the low nutrient density antibiotic free diets can improve the performance and immune status of broilers without affecting their carcass characteristics.

Key words: Broilers, Growth response, Humic acid, Immunity, Nutrient utilization

The use of most antibiotic growth promoters has been banned in many countries, because it is risky due to cross-resistance amongst pathogens and residues in tissues. Humates, one of the potential substances alternative to antibiotics in the diet of poultry, are formed from decayed plant matter with the aid of living bacteria in the soil. Humates include humus, humic acid, fulvic acid, ulmic acid, and trace minerals (Stevenson 1994), and on inclusion in feed and water of poultry promote growth (Bailey *et al.* 1996, Parks 1998, Shermer *et al.* 1998, Eren *et al.* 2000). Although the exact mode of action of the humic acids is not clear yet the addition to poultry diets improved nutrient digestibility, growth and food conversion efficiency. Humic acids stabilize the intestinal flora ensuring an improved utilization of nutrients in animal feed thus increase live weight of animals without increasing quantity of diet of animals (Humin Tech 2004). Humic acids are also able to

improve immune functions in the animal, reduce the incidence of diarrhea and other digestive upsets to a considerable extent (Humin Tech 2004). Pukhova *et al.* (1987) found that sodium humate increased the lifespan of mongrel rats exposed to lethal doses of cobalt radiation. In addition, humic substances also exhibit antiviral properties in particular against rhinovirus (Enviromate 2002). However, there are limited reports available on effect of humic acids on performance, nutrient metabolizability, carcass quality and immune status of broilers. In this context, a trial was undertaken to study the efficacy of a humic acid based feed supplement as a substitute for antibiotic in broilers.

MATERIALS AND METHODS

The humic acid preparation employed in this experiment was a commercial product. Day-old, straight run commercial broiler chicks (280) were wing banded, weighed and distributed randomly into 4 equal treatments with duplicate groups of 14 chicks each. Two basal diets, viz. moderate nutrient density diet as per the BIS (1992) specified ME and CP levels and low nutrient density diet containing 3.5% lesser ME and CP values than the BIS (1992) were formulated. Low and moderate nutrient density

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basal diets without added antibiotic were negative controls (T_1 and T_6) and with added antibiotic became positive controls (T_2 and T_7 , respectively). Further, the antibiotic free low (T_1) and moderate density diets (T_6) diets were supplemented with 0.5, 0.75 or 1g HA based product per kg of feed to result in test diets viz., T_3 , T_4 or T_5 and T_8 , T_9 or T_{10} , respectively. Each of such diets prepared for starter (0–14days), grower (15–28days) and finisher (29–42days) phases, were offered to duplicate groups of 14 chicks each.

The ingredient and calculated chemical composition of experimental diets prepared for different phases is presented in Table 1. Each of such 10 diets was offered to respective duplicate groups of birds housed in raised wire floor colony cages. On 22nd day, 4 birds from each replicate were sacrificed for ileal nitrogen absorbability studies and the experiment was continued with remaining 10 birds/replicate. Standard vaccination and managemental practices were followed uniformly. The feed and water were provided *ad lib*.

Experimental procedures: Body weight of individual broiler chicks and group feed intake of chicks in each replicate were recorded at weekly intervals. The feed conversion ratio was calculated on the basis of unit feed intake to unit gain in body weight for each replicate, separately.

A 3-day metabolic trial was conducted during grower phase (19th, 20th and 21st day) during which the daily feed intake and excreta voided in each group were recorded accurately. Care was taken to collect the excreta free from feed, feathers and scales.

On the 10th day of post vaccination for ND (day 17 of age),

about 2 ml of blood was collected separately in test tubes (non-heparinised) from 4 randomly selected birds in each replicate. Serum was collected after 8 to 10 h as per standard procedure (Calnek *et al.* 1992) and was stored in deep freezer at -20°C for subsequent analysis. Similarly blood was also collected from birds at 10-day post IBD vaccination (day 22 of age). The individual serum samples were assayed for antibody titre against ND by HI test and IBD using ELISA technique. Commercial test kits available for detection of antibodies against IBD were used for the purpose.

At the terminal day of experiment, 2 birds from each replicate (4 / treatment) were randomly selected, starved for 12 h with the provision of water *ad lib*. and weighed and sacrificed for carcass evaluation. Data in terms of weight of dressed chicken, breast meat and various organs, viz. liver, gizzard, heart, spleen, bursa, abdominal fat pad (g /100g pre-slaughter body weight) were recorded.

The net return was calculated by taking into account of the prevailing prices of constituent feed ingredients and additives including enzymes and sale price of live broiler birds. The performance index score (PIS) as the relationship of weight gain, per cent livability, FCR and number of days reared under each dietary group has been worked out and the PIS's relationship with cost of each diet as economic index score (EIS) was also arrived.

Statistical analysis: Data were subjected to analysis of variance (Snedecor and Cochran 1994). Comparison among means were made by Duncan's multiple range test (Duncan 1955) and significance was accepted at $P>0.05$.

Table 1. Per cent composition of experimental basal diets

Ingredients (kg)	Starter diet(0–14 days)		Grower diet (15–28 days)		Finisher diet (29–42 days)	
	Moderate ¹	Low ²	Moderate ¹	Low ²	Moderate ¹	Low ²
Maize	55.00	51.00	58.10	53.60	62.95	57.90
De-oiled rice bran	-	7.15	-	8.75	-	6.00
Soybean meal	40.15	34.80	36.05	32.30	30.70	25.05
Sunflower meal	-	-	-	-	-	5.00
Soya-lecithin oil	1.50	1.00	2.50	2.00	3.00	2.70
Di-calcium phosphate	1.80	1.80	1.80	1.80	1.80	1.80
Calcite	1.00	1.00	1.00	1.00	1.00	1.00
Trace minerals ³	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00
Additives ⁴	+	+	+	+	+	+
Chemical composition, %						
Dry matter	89.24	89.49	88.88	89.12	90.22	89.58
Crude protein	23.09	22.69	21.78	19.79	19.76	18.52
Total ash	7.45	7.83	7.91	7.71	6.72	7.87
Ether extract	4.18	4.04	4.89	4.24	5.35	5.34
Crude fiber	3.82	5.11	4.39	4.84	3.19	5.12
NFE	61.16	60.33	61.03	63.42	64.98	63.15
ME ⁵ , Kcal/kg	2,900	2,800	2,975	2,875	3,050	2,950

¹Moderate nutrient density diet; ²Low nutrient density diet; ³Trace mineral mix contained Fe, 90,000ppm; I, 2,000ppm; Cu, 15,000ppm; Mn, 90,000ppm; Zn, 80,000 ppm; Se, 300 ppm. ⁴Additives included commercial vitamin mix-0.05kg (each 500g contained vit A-,12.5 MIU; vit D₃, 2.8 MIU; vit E, 30g; vit K, 2g; vit B₁, 2g; vit B₂, 5g; vit B₆, 3g; vit B₁₂, 0.015g; niacin, 40g; calcium D panthothenate, 15g; folic acid, 1g; biotin, 0.08g; organic carrier.Q.S); tylosin phosphate, 0.05kg; hepatocare, 0.1kg; choline chloride, 0.05kg; curotox, 0.05kg; DL-methionine 0.5%. ⁵Calculated values.

Table 2. Performance of birds supplemented under different treatment levels during different phases of the experiment

Dietary treatments	No.	Body weight gain, g			Feed consumption, g			FCR, kg feed/kg weight gain		
		0-14days*	15-28days	29-42days	0-14days	15-28days	29-42days*	0-14days	15-28days*	29-42days
Low										
-	T ₁	262.4±3.20 ^{abc}	672.0±24.3	1013±59.6	1846±94.6	482.1±0.6	1146±10.4	1.84±0.04	1.98±0.04 ^{ab}	1.97±0.12
Antibiotic	T ₂	255.1±5.58 ^c	716.7±15.8	1128±35.4	2105±50.1	481.8±2.0	1157±62.8	1.86±0.04	1.98±0.01 ^{ab}	1.85±0.06
0.05% HA	T ₃	257.1±6.57 ^{bc}	659.2±29.3	1052±49.1	1971±70.3	482.0±1.0	1156±0.6	1.87±0.00	2.02±0.00 ^{ab}	1.87±0.05
0.075% HA	T ₄	263.0±5.93 ^{abc}	651.4±15.3	1111±40.3	2035±48.5	483.6±1.9	1177±8.7	1.83±0.00	2.09±0.00 ^a	1.82±0.06
0.1% HA	T ₅	267.0±5.04 ^{abc}	668.3±30.5	1114±25.9	2054±48.4	479.1±5.3	1160±10.1	1.78±0.05	2.01±0.01 ^{ab}	1.75±0.00
Moderate										
-	T ₆	271.0±6.73 ^{abc}	683.9±15.0	1072±35.2	2034±43.8	475.4±1.3	1180±20.4	1.77±0.01	2.00±0.01 ^{ab}	1.98±0.12
Antibiotic	T ₇	274.2±4.87 ^{abc}	720.3±26.2	1078±18.6	2071±45.3	475.2±2.0	1169±16.2	1.79±0.03	1.88±0.01 ^b	1.89±0.08
0.05% HA	T ₈	276.6±4.67 ^{abc}	693.5±17.9	1147±36.2	2125±43.7	476.0±2.7	1179±19.5	1.70±0.10	1.97±0.04 ^{ab}	1.74±0.13
0.075% HA	T ₉	274.1±7.43 ^{abc}	722.3±31.1	1039±56.8	2058±75.9	480.3±1.5	1128±8.1	1.73±0.01	1.87±0.10 ^b	1.96±0.01
0.1% HA	T ₁₀	284.0±5.49 ^a	700.7±25.1	1023±65.8	2008±69.2	481.5±2.1	1155±11.7	1.70±0.02	1.90±0.02 ^{ab}	1.91±0.03

*With in column, means bearing at least one common superscript are statistically similar (P>0.05).

RESULTS AND DISCUSSION

Body weight gain: During starter phase, the average body weight gains were significantly (P<0.05) different with the lowest value of 255.1 g/bird in low nutrient density diet supplemented with antibiotics and highest value of 284.0 g/bird in moderate nutrient density with 0.1% HA fed group. However, during grower and finisher phases, the body weight gains were statistically similar (P>0.05) among different groups. The cumulative average body weight gains were found to be similar in all the treatment groups indicating that the HA supplementation has no much role in body weight gain. Further, it was noticed that the HA supplemented groups were numerically lower than the antibiotic supplemented groups. The former statement was similar to the conclusion made by Bailey *et al.* (1996). Contrary to this, Kocabagli *et al.* (2002), Eren *et al.* (2000) and Teravita (2004) reported that the inclusion of HA improved the body weights of broilers. They also reported that the beneficial effect was more during later stages of growth.

Feed consumption: There were no significant (P>0.05) differences in feed consumption during starter and grower phases of the trial. However, during finisher phase as well as cumulatively, feed consumption varied significantly (P<0.05). The highest cumulative feed consumption was recorded in birds fed with low nutrient density diet supplemented with 0.075% HA as against the lowest of 3102 g/bird in birds fed with antibiotic based moderate nutrient density diet. There was an inconsistent variation in the feed consumption as the level of HA in their diets gradually increased. In general, feed consumption was more in birds fed on low nutrient density diets compared to moderate nutrient density diet fed birds as the nutrients prevailing at low levels renounced higher feed consumption. The results indicated that the feed consumption was affected by supplementation of HA signifying that the HA does affect bird's appetite. These findings are in agreement with the reports of Kocabagli *et al.*, (2002) and Eren *et al.* (2000) that the HA supplementation does not alter the feed intake but stabilizes the gut microflora ensuring better nutrient utilization. Similarly, Kucukerasan *et al.* (2005) reported the feed consumption decreased nonsignificantly with increasing level of inclusion of HA but improved the egg production in layers.

Feed efficiency: The feed conversion ratio (FCR) was nonsignificant (P>0.05) during starter and finisher phases of experiment as well as cumulatively (Table 2). However, the FCR values were significantly different for grower phase with better FCR of 1.87 (moderate density + 0.075% HA) and poorer of 2.02 (low density + 0.05% HA). The overall cumulative FCR was lowest in 0.1% HA supplementation (1.93) and highest in antibiotic free control diet (2.04) among low nutrient density diets, while better in 0.05% HA based diet (1.90) and poorer in antibiotic free control (2.01) among moderate nutrient density diets. The cumulative response of the birds was numerically better in HA supplemented groups when compared to both the antibiotic free and antibiotic included groups. Feed

efficiency was enhanced with the supplementation of Humic Acids, however not consistent with the level of inclusion either in the low nutrient or moderate nutrient density diets. Similar results were observed by Bailey *et al.* (1996), Eren *et al.* (2000), Ceylan and Ciftci (2002), Kocabagli *et al.* (2002) and Islam *et al.* (2005) who opined that the supplementation of HA improves FCR in broilers. This conclusion is also supported by Parks (1998) that the HA supplementation improves FCR significantly in turkeys.

Metabolizability of nutrients: The per cent metabolisability of dry matter and crude protein values (Table 3) under different treatments were statistically ($P < 0.05$) different. At most of levels of HA incorporation better DM metabolisability was observed than controls, which could be attributable to the improved utilization of nutrients including minerals. Similarly, trend was also observed in CP metabolisability. However, no significant differences were observed among different treatments in organic matter, ether extract, crude fiber and NFE metabolisability. The average ileal nitrogen absorbability of birds (Table 3) under different treatments were also remained non-significant, however were numerically superior in HA supplemented diets. This implies that HA increases the uptake of nitrogen due to their chelating effects. The results are in agreement with the findings of

Shermer *et al.* (1998) and Humin Tech (2004) who hypothesized that humates might increase the uptake of nitrogen due to their chelating effects.

Carcass characteristics: The dressing percentage and breast meat yield values (Table 4) among different treatments were statistically ($P > 0.05$) similar indicating that HA at different levels has no influence on dressing percentage and breast meat yield. Similarly, the abdominal fat content values were also similar statistically ($P > 0.05$) implying that abdominal fat pad of broilers was not influenced by HA supplementation. This implies that the supplementation of HA at different levels has no influence on dressing percentage and abdominal fat, which were in conformation with the findings of Kocabagli *et al.* (2002). However, Parks (1998) demonstrated that the HA supplementation at 0.1% level significantly decreases the fat pad weights in turkeys.

The relative weights of liver, heart, spleen, bursa and gizzard (Table 4) under different treatments were not affected significantly by HA supplementation. The overall liver mass and weight of the organs heart, spleen and bursa were better in HA supplemented groups compared with antibiotic control groups, whereas of the weight of the organ gizzard was does not affected by HA supplementation. However, no literature is available to confirm the same.

Table 3. Metabolisability of various nutrient and ileal N absorbability (%) of diets under different treatments

Nutrient density	Feed additive	Tr.No.	Dry matter*	Organic matter	Crude protein*	Ether extract	Crude fiber	NFE	Ileal N absorbability
Low	-	T ₁	64.83±0.8 ^{ab}	68.16±0.9	49.84±5.8 ^{ab}	71.08±1.2	68.32±0.4	73.68±3.1	75.26±4.00
	Antibiotic	T ₂	65.53±0.5 ^{ab}	68.85±0.2	45.64±0.4 ^{ab}	77.57±5.3	67.72±2.4	75.59±0.0	84.28±0.19
	0.05% HA	T ₃	64.52±1.2 ^b	67.99±1.1	45.19±0.1 ^{ab}	67.11±1.9	67.01±4.0	75.23±1.2	93.22±0.03
	0.075% HA	T ₄	65.93±0.8 ^{ab}	69.31±0.7	49.78±1.9 ^{ab}	73.71±0.2	73.41±0.0	74.80±0.4	96.42±0.30
	0.1% HA	T ₅	64.76±0.9 ^{ab}	68.39±0.9	41.14±0.4 ^b	71.60±1.0	73.55±0.0	76.28±1.2	96.20±0.52
Moderate	-	T ₆	68.50±0.4 ^{ab}	71.35±0.5	55.49±0.2 ^a	77.20±0.9	73.05±0.8	76.42±0.9	86.60±0.55
	Antibiotic	T ₇	66.99±0.2 ^{ab}	69.88±0.4	56.53±3.3 ^a	74.88±0.7	70.36±0.6	74.21±1.8	86.21±0.29
	0.05% HA	T ₈	68.53±0.6 ^a	71.21±0.3	57.84±2.8 ^a	73.15±3.2	69.17±2.1	75.98±1.4	94.45±0.41
	0.075% HA	T ₉	67.77±0.7 ^{ab}	70.60±0.7	50.68±0.2 ^a	74.59±1.5	69.49±1.6	77.47±1.2	96.26±0.11
	0.1% HA	T ₁₀	68.04±0.2 ^{ab}	70.74±0.4	55.19±0.5 ^a	74.32±0.4	68.92±2.5	76.13±0.6	96.03±0.03

*With in column, means bearing at least one common superscript are statistically similar ($P > 0.05$).

Table 4. Carcass characteristics and relative weight of vital organs (g/100g live wt.) under different treatments

Nutrient density	Feed additive	Tr.No.	Dressing percentage	Breast meat yield	Heart	Liver	Spleen	Bursa	Gizzard fat	Abdominal fat
Low	-	T ₁	65.02±0.1	15.31±0.41	0.59±0.01	1.89±0.14	0.23±0.01	0.41±0.18	2.39±0.24	0.86±0.21
	Antibiotic	T ₂	66.65±1.0	16.52±1.44	0.50±0.03	1.53±0.03	0.12±0.00	0.26±0.01	2.36±0.07	0.75±0.04
	0.05% HA	T ₃	66.83±1.4	15.76±1.53	0.55±0.04	1.66±0.07	0.19±0.37	0.25±0.04	2.26±0.39	0.66±0.17
	0.075% HA	T ₄	65.17±1.5	13.82±0.75	0.57±0.05	1.81±0.02	0.15±0.00	0.36±0.04	2.21±0.20	0.72±0.25
	0.1% HA	T ₅	66.07±0.6	14.66±0.23	0.47±0.17	1.73±0.33	0.14±0.04	0.24±0.18	2.61±0.68	0.69±0.18
Moderate	-	T ₆	66.82±1.9	15.17±1.86	0.63±0.05	1.82±0.01	0.19±0.01	0.37±0.05	2.41±0.15	0.88±0.09
	Antibiotic	T ₇	66.82±0.3	11.26±4.20	0.51±0.02	1.62±0.03	0.10±0.03	0.22±0.03	2.65±0.07	0.87±0.11
	0.05% HA	T ₈	62.69±0.8	14.59±1.85	0.55±0.04	1.70±0.05	0.16±0.01	0.28±0.04	2.56±0.38	0.66±0.17
	0.075% HA	T ₉	64.10±1.6	15.96±1.15	0.46±0.03	1.79±0.00	0.12±0.02	0.27±0.11	2.41±0.06	0.79±0.06
	0.1% HA	T ₁₀	67.13±0.1	16.65±0.49	0.61±0.00	1.78±0.15	0.17±0.05	0.25±0.02	2.39±0.00	1.04±0.19

Livability: The percentage livability of birds (Table 3) under different treatments during the 42 days experimental period was statistically ($P>0.05$) similar. The livability value of 97.5% was found in birds fed on either low or moderate density antibiotic based diets and also in moderate density diets with 0.075% HA. Whereas the livability of remaining groups was 100%, indicating that HA decreased the mortality and unspecific deaths. The study indicated that the addition of HA decreased mortality and the results were in agreement with Kocabagli *et al.* (2002) and Islam *et al.* (2005) who reported that the incorporation of humates in the feed of chickens reduced unspecific deaths. However, Bailey *et al.* (1998) reported that the HA supplementation increased the mortality significantly.

Antibody titer against ND and IBD: The mean HI titer values for Newcastle disease (Table 5), though statistically ($P>0.05$) nonsignificant among different treatments, yet numerically better titer values were observed with HA supplementation. The mean ND titer values ranged from 108 (low; Ab control) to 336 (moderate; 0.1% HA) implying that RD titer values. The mean IBD titer values of birds under different treatments were statistically ($P<0.05$) significant, ranging from as low as 165.8 (low; Ab free control) to as high as 1999 (low; 0.075% HA) implying the mean IBD titer values were statistically ($P<0.05$) significant. The results were in correlation with the observation made by Lenk and Benda (1989) and Gribon *et al.* (1991), who reported an improved immune system of

calves by HA supplementation. Hence, HA supplementation was found to enhance the antibody production against IBD in a better way than antibiotic free or antibiotic supplemented groups.

Economics of HA supplementation: The net profit per bird (Table 6) among different treatment groups were statistically nonsignificant ($P>0.05$) and were ranging from ₹ 7.16 in antibiotic free moderate nutrient density diet fed groups to ₹ 11.96 in 0.1% HA supplemented low nutrient density diet fed groups. The HA supplemented diets showed better net profit than unsupplemented control groups and in many cases net returns in HA supplemented groups were better than antibiotic supplemented groups, since Humic preparations are cheaper than antibiotics, besides improving broiler performance. Although the PIS and EIS values (Table 4) were statistically similar ($P>0.05$) among different treatments, the HA supplemented diets showed numerically better scores than antibiotic supplemented or unsupplemented dietary groups. This indicated that supplementation of HA preparation was beneficial and can replace the antibiotics economically.

Based on different parameters studied, it was concluded that the addition of HA up to 0.1% particularly in low nutrient density antibiotic free diets can improve the performance and immune status of broilers without affecting the carcass characteristics. However, further studies are required to evaluate beneficial effects of HA to replace antibiotics in poultry rations.

Table 5. Livability and antibody titer values of birds against ND and IBD under different treatments

Nutrient density	Feed additive	Tr. No.	Livability%	HI ND titer	ELISA IBD Titer*
Low	-	T ₁	100.0±0.0	116±28.75	165.8±82.06 ^d
	Antibiotic	T ₂	97.5±2.5	108±23.89	222.5±94.13 ^{cd}
	0.05% HA	T ₃	100.0±0.0	272±54.65	1875.0±512.90 ^a
	0.075% HA	T ₄	100.0±0.0	280±68.35	1999.0±569.10 ^a
	0.1% HA	T ₅	100.0±0.0	260±55.57	536.4±127.00 ^{abcd}
Moderate	-	T ₆	100.0±0.0	122±15.79	455.1±66.82 ^{bcd}
	Antibiotic	T ₇	97.5±2.5	344±68.35	1049.0±221.50 ^{abcd}
	0.05% HA	T ₈	100.0±0.0	264±72.00	557.1±79.25 ^{abcd}
	0.075% HA	T ₉	97.5±2.5	208±61.97	992.1±353.80 ^{abcd}
	0.1% HA	T ₁₀	100.0±0.0	336±48.00	624.8±87.25 ^{abcd}

*With in column, means bearing at least one common superscript are statistically similar ($P>0.05$).

Table 6. Economics of broilers under different treatments

Nutrient density	Feed additive	Tr.No.	Net returns (₹/bird)	PIS	EIS
Low	Ab free control	T ₁	9.69±1.07	215.0±7.2	20.41±0.68
	Ab control	T ₂	10.74±1.25	220.9±20.8	20.72±1.94
	0.05% HA	T ₃	9.96±0.50	218.8±4.3	20.65±0.41
	0.075% HA	T ₄	10.77±0.85	229.3±7.3	21.58±0.69
	0.1% HA	T ₅	11.96±0.24	238.9±0.9	22.42±0.09
Moderate	Ab free control	T ₆	7.16±1.30	210.2±17.2	18.78±1.54
	Ab control	T ₇	8.30±0.48	217.2±9.8	19.20±0.87
	0.05% HA	T ₈	11.03±2.70	252.6±27.3	22.45±2.43
	0.075% HA	T ₉	7.74±0.04	215.3±10.2	19.09±0.91
	0.1% HA	T ₁₀	8.26±0.54	227.0±6.1	20.07±0.54

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