PERFORMANCE, HEMATOLOGICAL PROFILE AND SERUM BIOCHEMICAL COMPOSITION OF BROILER CHICKENS FED DIETS WITH DIFFERENT SALT LEVELS

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Abstract
This study evaluated the effect of different dietary salt levels on broilers' performance, hematological profile and serum biochemical composition. A total of one hundred and forty-four (144) day-old chicks were used. The birds were divided into four treatment groups, each subdivided into three replicates with twelve (12) birds per replicate. Four experimental diets were formulated (T_1, T_2, T_3, T_4). T_1 (control) contains 0.00% common salt level while T_2, T_3, and T_4 contained 0.25, 0.50, and 0.75 % common salt, respectively, both at the starter and finisher phases. Each treatment group of birds was assigned to one of the four (4) experimental diets in a completely randomized design. The experiment lasted for nine weeks. Data were collected on feed intake and weight gain. Blood samples were taken for hematological and serum biochemical analyses. Birds on T_4 had the highest ($p<0.05$) average live weight (1982.60 g) while those on the 0.00 % salt diet had the lowest (1676.60 g). Birds on T_1 had a higher ($p<0.05$) feed conversion ratio (4.60) while those on T_3 had the least value (3.50). Significant differences ($p<0.05$) in blood counts were observed among treatments except for MCV, MCH, MCHC, and WBC. Birds on T_3 had higher Hb, PCV, RBC and platelets counts and were closely followed by birds placed on T_4 diet. Results of serum biochemical analysis showed significant differences ($p<0.05$) among treatments. Birds on T_4 had higher Na^+ and Cl^- while birds on T_1 had higher Fe^{3+}, Mg^{2+} and uric acid contents. Birds on T_1 had higher triglycerides and were closely followed by birds on T_4. Birds on T_4 had higher aspartate aminotransferase levels compared to others. It was concluded that increasing salt levels up to 0.75% in a broiler diet brought about improvements in live weight gain and had no negative effect on hematological and serum biochemical indices.
Keywords: Broiler Chicken, Performance, Salt, Hematology, Serum Biochemical Indices.

Introduction

Sodium is an essential nutrient known to influence several aspects of animal growth. It affects water intake, acid-base balance (Jiang *et al*., 2019; Wang *et al*., 2020) and membrane potentials of cells (Mushtaq *et al*., 2013). Common salt is the most important source of sodium, costs little and provides chloride and other essential elements such as iodine, vitamin A, etc. Sodium and Chloride, along with potassium, are the electrolytes required in greatest amounts by birds, and they are required in the preservation of the cellular membrane electrochemical gradient (Lata & Mondal, 2021). National Research Council (NRC,1994) recommended 0.20% and 0.15% of sodium chloride from 0-3 and 4-6 weeks of age, respectively.

In intensively raised fast-growing chickens an adequate intake of dietary sodium has a beneficial influence on feed consumption and the growth rate of birds (Borges *et al.*, 2004). The provision of adequate levels of the other major minerals in appropriate balance is also very important in the successful rearing of broilers and for optimal performance (Yu *et al*., 2017).

Sodium is involved in the regulation of acid-base balance, osmotic pressure, and protection against excessive loss of body fluid (Wang *et al*., 2019). The permeability of cells and the sustenance of normal muscle irritability are additional functions of sodium (Nobakht, 2005). Sodium and Chloride are involved in the regulation of bone development and mineralization (Araujo *et al*., 2022). Sodium is also needed for absorption of amino acids and sugars in the small intestine. Therefore, the utilization of digested protein and carbohydrates is diminished with an insufficiency of this cation (Mushtaq, 2010). Likewise, both sodium and chloride aid in nutrient passage and waste removal in cells. Sodium deficiency leads to reduced growth and feed consumption and impairs feed conversion (Ross, 1979). Dietary deficiency of sodium results in acid-base imbalance, reduced cardiac output, growth reduction, increased feed conversion ratios, gonadal dormancy, bone softening, corneal keratinization, decreased cellular volume, and changes in cellular function (Mushtaq, 2010). Adrenal malfunction which may lead to increased uric acid levels, shock, and ultimately death has been linked to sodium deficiency in chicks (Leeson & Summers, 2001). In chickens, sodium homeostasis in blood and tissue is maintained by excreting excess sodium or retention when there is dietary inadequacy (Lesson & Summers, 2001). Though NRC (1994) recommended 0.15-0.20% dietary levels, the growth performance of birds improved when the sodium content of the feed was increased from 0.20 to 0.30% (Oviedo-Rondo *et al.*, 2001: Mushtaq *et al*., 2007). An increase in dietary sodium to 0.30% is reported to improve breast muscle yield and reduce abdominal fat deposition (Mushtaq *et al*., 2005).

On the other hand, a dietary increase in sodium chloride content has been reported to increase litter moisture content. Litter dampness is correlated with the amount of sodium and chloride in the diet. This led to recommendations to minimize dietary levels (Borges *et al.,* 2004; Mushtaq *et al.,* 2005; 2007; Vieira *et al*., 2003). High moisture content in litter increases the risk of diseases such as foot pad dermatitis (Juszkiewicz *et al*., 2009). However, this may be of little importance for a pre-starter feed, considering the small amount of feed intake and excreta produced in this period (NRC, 1994). Secondly, the high ambient temperatures in the tropics, vis-a-vis the cooler temperatures in the temperate regions, greatly increase moisture evaporation, thereby minimizing litter dampness. Thirdly, the dietary requirements of broiler chickens should be reexamined regularly because the continuous genetic selection for improvement in growth rate alters their genetic and physiological makeup. Dietary requirements established decades ago may not be appropriate today. Therefore, the objective of this study was to re-evaluate the optimal dietary inclusion level of salt in broiler diets based on effects on performance in addition to hematological and biochemical profiles.

Materials and Method

Experimental Diets

Four broiler starter and four broiler finisher diets were formulated. Diets 1, 2, 3, and 4 were formulated to contain 0.00%, 0.25%, 0.50% and 0.75% common salt (NaCl), respectively. The details are shown in Tables 1 and 2. Broiler starter diets were fed from 0-4 weeks while broiler finisher diets were fed from 5-8 weeks of age.

Table 1: Ingredient composition of the starter diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Maize	54.00	53.75	53.50	53.25
Soya bean meal	21.75	21.75	21.75	21.75
Palm kernel cake	10.00	10.00	10.00	10.00
Wheat offal	5.00	5.00	5.00	5.00
Bone meal	3.00	3.00	3.00	3.00
Lysine	0.60	0.60	0.60	0.60
Methionine	0.40	0.40	0.40	0.40
Vit|TM	0.25	0.25	0.25	0.25
Salt	0.00	0.25	0.50	0.75
Total	100	100	100	100
Calculated values(%)				
ME (Kcal/kg)	2833	2824.5	2816	2807
Crude protein	21.25	21.25	20.98	20.96

*Provides the following per kg of feed: Vitamin-mineral premix (2.5 kg/1000 kg); vitamin A (10,000,000 IU), vitamin D3 (3,000,000 IU), vitamin E (30,000 IU), vitamin K (2.3 g), vitamin B1 (2.0 g), Riboflavin (5.0 gr), Pyridoxine (3.0 g), vitamin B12 (160 mg), Biotin (60 mg), Niacin (31 g), panthotenic acid (8 g), folic acid (1 g), manganese (85 g), zinc (50 g), iron (25 g), copper (6 g), iodine (1 g), selenium (120 g), cobalt (220 mg), antioxidant (125 g), choline chloride (200 g).

Table 2: Ingredient composition of the finisher diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Maize	50.75	50.50	50.25	50.00
Soya bean meal	18.00	18.00	18.00	18.00
Palm kernel cake	18.00	18.00	18.00	18.00
Wheat offal	5.00	5.00	5.00	5.00
Fish meal	4.00	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00	3.00
Lysine	0.60	0.60	0.60	0.60
Methionine	0.40	0.40	0.40	0.40
***Premix**	0.25	0.25	0.25	0.25
Salt	0.00	0.25	0.50	0.75
Total	100	100	100	100
Calculated values(%)				
ME (Kcal/kg)	2820	2812	2803.5	2795
Crude protein	19.46	19.44	19.41	19.39

***** Provides the following per kg of feed: Vitamin-mineral premix (2.5 kg/1000 kg); vitamin A (10,000,000 IU), vitamin D3 (3,000,000IU), vitamin E (30,000 IU), vitamin K (2.3 g), vitamin B1 (2.0 g), Riboflavin (5.0 gr), Pyridoxine (3.0 g), vitamin B12 (160 mg), Biotin (60 mg), Niacin (31 g), panthotenic acid (8 g), folic acid (1 g), manganese (85 g), zinc (50 g), iron (25 g), copper (6 g), iodine (1 g), selenium (120 g), cobalt (220 mg), antioxidant (125 g), choline chloride (200 g).

Experimental Design and Management of Birds

A total of one hundred and forty-four (144) day-old unsexed broiler chicks were used in the experiment The birds were randomly divided into four treatment groups with three replicates of 12 birds each in a completely randomized design (CRD). Each treatment group was assigned randomly to one of the experimental diets. All replicates were housed in pens of similar dimensions (4 x 4 feet). Feed and water were provided *ad libitum* for the period of the experiment. Fresh and clean wood shavings were used as litter material over a concrete floor and changed regularly. All necessary vaccinations, medications and other management practices were administered to the birds following conventional practice specified in the guidelines produced by the Management of the Teaching and Research Farm, Federal University of Technology, Owerri.

Determination of Feed Intake, Weight Gain and Feed Conversion Ratio

Data was collected on daily feed intake, daily live weight gain, and feed conversion ratio on a replicate basis. Daily feed intake was obtained as the difference between the weights of leftover feed in the morning and the weight of the feed offered the previous morning. The daily feed intake was averaged over the feeding trial period which gave the average daily feed intake. The birds were weighed individually at the end of each week. The difference between the initial and final weights of the birds was taken as the weight gain which was averaged within each treatment to give the average daily weight gain (ADWG). The feed conversion ratio(FCR) was calculated as the average daily feed intake (ADFI) divided by the average daily weight gain (ADWG). Feed cost/kg was calculated by summing the cost of feed ingredients per 100 kg of feed and dividing it by 100. Feed cost per kg weight gain was calculated by multiplying the feed cost per kg by the feed conversion ratio.

Blood Collection and Analyses

At the end of the feeding trial, from each replicate, two birds whose live weights were closest to the mean live weight of that replicate were randomly selected. The birds were starved of food but not water for 24 hours. The liveweight of each bird was determined, and thereafter it was slaughtered by severing the jugular vein and bled in a vertical position. Two ml of blood was collected into sample bottles treated with ethylenediaminetetraacetic acid (EDTA) and used for the determination of blood counts as outlined by Uko *et al*. (2000). Another 2 ml was collected into plain Bijou bottles for assessment of serum biochemical composition as outlined by Kohn & Allen (1995) and Mouldin *et al.* (1996).

Statistical Analysis

Data collected on performance, hematological profile, and serum biochemical composition were subjected to analysis of variance for the completely randomized design (CRD). Significantly different means were separated using the least significant difference (LSD) test. The Minitab Statistical Package was used.

Results and Discussion

The result of the growth performance of broilers at the starter phase is presented in Table 3. There were significant differences (p<0.05) in live weight, weight gain, feed intake, and feed conversion ratio between the birds fed with feed containing different salt inclusion levels both at the starter and finisher phases.

Table 3: Performance of broilers fed diets containing different salt inclusion levels at the starter phase.

Parameter	0.00%	0.25%	0.50%	0.75%	SEM
Initial weight (g)	44.70^{ab}	45.67^{a}	44.30^{ab}	42.73^{c}	0.29
Final weight (g)	463.30^{c}	651.45^{a}	403.40^{c}	575.80^{b}	21.61
Body weight gain (g/bird)	418.60^{b}	605.33^{a}	359.10^{c}	533.07^{a}	21.32
Average daily weight gain (g/bird)	59.80^{b}	86.46^{a}	51.30^{c}	76.15^{a}	3.05
Average feed intake (g/bird/day)	84.25^{c}	106.77^{a}	94.22^{b}	101.71^{a}	1.80
Feed conversion ratio	1.41^{a}	1.23^{c}	1.33^{b}	1.34^{b}	0.59

^{abc} Means within the same row bearing different superscripts are significantly different (P<0.05).

Birds fed diets containing 0.25% common salt were significantly higher (p<0.05) in live weight than those fed 0.75% 0.50% and 0.00% salt inclusion diets. This is in line with the recommendations of NRC (1994) which stipulates 0.20% and 0.15% sodium chloride inclusion level from 0 - 3 and 4 – 6 weeks of age. Birds-fed diets containing 0.25% and 0.75% salt inclusion levels were similar in feed intake but differed significantly from birds-fed diets containing 0.50% and 0.00% salt inclusion. Feed intake was lowest in birds fed 0.00% salt inclusion. The reduced feed consumption may be due to the unpalatability of the feed or may have a physiological underpinning. Feed conversion ratio (FCR) was significantly higher (p<0.05) in birds fed 0.00% than in counterparts fed 0.25%, 0.50% and 0.75% salt inclusion diets.

Table 4: **Performance of broilers fed diets containing different salt inclusion levels at the finisher phase.**

Parameter	0.00%	0.25%	0.50%	0.75%	SEM
Initial body weight (g/bird)	463.30^{c}	651.50^{a}	403.40^{c}	575.80^{b}	9.36
Final body weight (g/bird)	1499.10^{c}	2075.10^{a}	1862.80^{b}	2095^{a}	20.85
Body weight gain (g/bird)	1035.80^{c}	1423.60^{b}	1459.40^{b}	1519.2^{a}	11.49
Average daily weight gain (g/bird)	29.77^{c}	40.68^{b}	$41.70b^{a}$	43.26^{a}	0.47
Average feed intake (g/bird/day)	138.42^{c}	158.10^{a}	148.41^{b}	162.46^{a}	0.86
Feed conversion ratio	4.60^{a}	3.80^{b}	3.50^{c}	3.70^{bc}	0.04
Feed cost (#/kg)	88.41	89.71	89.71	89.28	
Feed cost/ kg weight gain	259.34	247.57	245.63	250.85	3.33
Mortality (%)	0.00	1.00	1.75	1.25	0.11

^{abc} Means within the same row bearing different superscripts are significantly different (P<0.05).

Results presented in Table 4 showed that birds fed the diet containing 0.75% salt were highest in the final weight, weight gained, and average daily weight gain ($p<0.05$) than counterparts fed 0.25%, 0.50% and 0.00% salt inclusion diets. Oviedo-Rondon *et al*. (2001) reported that the growth performance of birds was improved when the sodium content of feed was increased from 0.2% to 0.3%. Also, Britton, (1990, 1991), Zarnado, (1994) and Borges *et al*. (2004) observed better performance in birds when the sodium chloride content of their feed was increased from 0.20% - 0.73%. In support, Maiorka *et al*. (2004) and Ribeiro et al. (2008) recommended a 0.40% salt inclusion level as optimal for water consumption, feed intake and body weight gain. In particular, this study demonstrated that rearing birds on a salt-free diet is detrimental to performance, as the diet did not meet the nutrient requirements of birds for sodium and chloride. This finding is in agreement with Jankowski *et al*. (2011). Sodium has been reported to play an active role in nutrient transport across the intestinal wall (Gal-Garber *et al*. 2003). In addition, the difference in sodium chloride requirements of birds is believed to be related to the production potential of different strains of broilers.

The feed conversion ratio (FCR) improved with the increase in salt inclusion level until 0.50% inclusion ($p<0.05$). No significant differences ($p>0.05$) in FCR were found between those on 0.50% salt inclusion (3.50) and 0.75% salt inclusion levels (3.70). Birds on 0.00% salt inclusion level had the highest feed conversion ratio. Jankowski *et al*. (2011) reported that dietary sodium chloride higher than 1.69-2.61% did not improve the feed conversion ratio. In another study, Mushtaq (2010) reported that a sodium-deficient diet will lead to growth reduction and increased feed conversion ratio.

Hematological Properties

Farm animals' qualitative development, homeostasis, and viability are determined by the optimization of blood parameters (Azarnova *et al*., 2023). Also, they reflect the physiological responsiveness of the animal to its environment and thus serve as a veritable tool for monitoring animal health (Pascalonpekelniczky *et al*., 1994, 1996). Table 5 shows the effect of dietary salt inclusion levels on the hematological profile of broilers. Significantly different means were found for hemoglobin (HB), packed cell volume (PCV), red blood cell count (RBC), platelets, and differential white cell counts. Birds fed 0.50 % dietary salt level had significantly higher HB, PCV and platelet counts than those fed 0.00 % and 0.25 % dietary salt levels. Though the values for birds fed the 0.75 % salt level were numerically higher than the 0.00 % and 0.25 % dietary salt levels, the values were not significantly different from the values for birds on 0.00 % and 0.50 % inclusion levels. However, all the values were within the reference values reported for growing broilers (Wakenell, 2010; Bounous & Stedman, 2000). The higher HB, PCV, RBC and platelet counts of birds on 0.50% indicate that this level stimulated higher physiological activity.

No significant differences were found for MCV, MCH, and MCHC. These values were also within the reference values reported for healthy growing broilers by Wakenell (2010). This suggests that irrespective of the inclusion level of salt, the birds had normocytic and normochromic red cells, indicating that dietary inclusion of salt up to 0.75 % did not affect iron utilization within the conventional rearing period of broilers. The total white blood counts did not vary significantly ($P>0.05$) among treatment groups and the values were within the normal range reported by Wakenell (2010). Significant differences ($p<0.05$) were found in some differential WBC counts. Birds on 0.25 % dietary salt level had significantly lower lymphocyte counts than the birds on 0.00 % dietary salt level. Birds fed diets containing 0. 25 % salt level had higher neutrophils than their counterparts. Monocytes, basophils, and eosinophils were not observed in this study. It could be that these blood components were present in trace to small numbers and so were undetectable by the analytical procedure adopted. Howbeit, from the hematological characteristics of the experimental birds, it can be deduced that the birds were healthy as all the parameters measured were within the normal range.

The serum biochemical composition is shown in Table 6. All the values fell within the normal range reported by Dukes (1975) and Swenson (1970) for broiler chickens. These biochemical indices were not adversely affected by increasing the salt content of the diet up to 0.75%. However, there were significant differences ($p<0.05$) in some serum electrolytes like chloride, sodium, iron and magnesium. Chloride and sodium levels in birds fed the 0.75% salt diet were significantly higher ($p<0.05$) than the birds fed the 0.00 % salt diet.

Table 5 Effect of different salt inclusion levels on the hematological indices of broilers.

Parameter	0.00%	0.25%	0.50%	0.75%	SEM
Hemoglobin(g/dl)	9.00^{b}	9.42^{b}	10.67^{a}	9.82^{ab}	0.29
PCV (%)	28.12^{b}	29.53^{b}	32.97^{a}	30.12^{ab}	0.95
RBC ($\times 10^6$ul)	2.07^{b}	2.22^{ab}	2.44^{a}	2.27^{ab}	0.08
MCV (fl)	136.35	132.50	138.27	133.70	3.83
MCH (pg)	38.48	38.48	39.37	38.13	0.50
MCHC (g/dl)	28.33	29.05	28.32	28.67	0.58
Platelets ($\times 10^3$ul)	14.33^{b}	15.00^{b}	22.28^{a}	17.55^{ab}	1.67

WBC (×10³ul)	78.95	68.95	78.67	78.77	3.43
Lymphocytes (%)	87.50[a]	79.33[b]	86.00[ab]	86.17[ab]	2.05
Neutrophils (%)	12.50[b]	20.67[a]	12.33[b]	12.83[b]	1.89
Monocytes (%)	-	-	-	-	-
Basophils (%)	-	-	-	-	-
Eosinophils (%)	-	-	-	-	-

[abc] Means within the same row bearing different superscripts are significantly different (P<0.05)
PCV – Packed Cell Volume, RBC – Red Blood Cell, MCV – Mean Corpuscular Volume,
MCH – Mean Corpuscular Hemoglobin, MCHC – Mean Corpuscular Hemoglobin Concentration, WBC – White Blood Cell.

Table 6: Effect of different salt inclusion levels on serum biochemical components of broilers

Parameter	0.00%	0.25%	0.50%	0.75%	SEM
Cl^-(mmol/l)	106.10[b]	112.27[ab]	109.47[ab]	120.45[a]	3.62
Na^+(mmol/l)	139.15[b]	139.87[b]	143.78[ab]	152.70[a]	3.38
K^+(mmol/l)	4.53	4.56	4.80	4.96	0.38
Fe^{3+}(mmol/l)	91.17[a]	76.85[b]	66.08[c]	60.32[c]	2.19
Mg^{2+}(mg/dl)	1.33[a]	0.98[b]	1.05[ab]	0.95[b]	0.10
Ca^{2+}(mg/dl)	8.41	8.55	9.89	10.14	0.67
Inorganic phosphate(mg/dl)	2.68	2.48	2.90	3.10	0.26
Hco_3^- (mmo/l)	28.33	28.00	28.00	28.33	0.79
Urea(mg/dl)	11.27	6.48	6.39	6.30	1.94
Uric acid(mg/dl)	6.92[a]	5.62[ab]	6.15[ab]	4.90[b]	0.42

[abc] Means within the same row bearing different superscripts are significantly different (P350.18<0.05)

Table 7: Effect of different dietary salt levels on serum metabolites and enzymes of broilers

Parameter	0.00%	0.25%	0.50%	0.75%	SEM
Glucose (mg/dl)	226.17	160.58	172.42	152.02	23.92
Cholesterol (mg/dl)	100.21	162.49	144.85	115.43	24.96
Triglyceride (mg/dl)	127.15[a]	101.99[ab]	93.69[b]	126.19[a]	9.17
Total bilirubin (mg/dl)	0.11	0.09	0.21	0.21	0.07
Conj. bilirubin (mg/dl)	0.04	0.03	0.12	0.13	0.05
Creatine (mg/dl)	1.37	1.27	0.25	0.25	0.65
ALT (iu/l)	13.50	11.33	11.33	14.67	1.96
AST (iu/l)	255.70[b]	441.20[a]	405.70[ab]	526.30[a]	44.96
ALP (iu/l)	1846.50	2115.5	1767.80	2366.3	350.18

[abc] Means within the same row bearing different superscripts are significantly different (P<0.05)
ALT – Alanine aminotransferase, AST – Aspartate aminotransferase, ALP – Alkaline phosphate.

This is expected since a correlation is known to exist between dietary levels of electrolytes and serum components of chickens (Wakenell, 2010). Iron and magnesium were significantly highest ($p < 0.05$) in birds on the 0.00% salt diet, and the values decreased progressively with increase in dietary level of salt. This suggests a negative correlation between dietary salt inclusion, and serum iron and magnesium concentrations.

There were significant differences ($p<0.05$) in serum triglyceride level between the birds (Table 7). However, the values obtained in this study were within the normal range of serum triglycerides (121.57-127.88mg/dl) reported by Owosibo *et al.* (2013). Birds fed the 0.00 % and 0.75% salt inclusion diets were significantly higher in serum triglyceride levels than their counterparts. This result was unexpected because birds fed 0.00 % salt level were low in feed intake and also low growth performance. More so, the fat contents of 0.00% dietary salt inclusion birds were low compared to the other treatment groups (Table 7). It is possible however, that the reason for the reduced performance of birds on 0.00% dietary salt may be difficulty in mobilizing tissue fat. This means that Na and Cl may be implicated in mobilizing fats between tissues in the body.

There were significant differences (p<0.05) in uric acid levels between the treatments. The birds fed diets with 0.75% salt level had significantly (p < 0.05) lower uric acid content than the birds on 0.00 % salt level. The values decreased progressively with an increase in dietary salt level. It seems that the birds fed diets with 0.00 % salt inclusion were less able to utilize the protein from their feed which resulted in higher output of metabolic nitrogen. Another explanation may be that higher dietary salt levels would stimulate higher water intake resulting in hematological dilution. Consequently, body reserves were metabolized to maintain essential metabolic activities. This will result in lowered body weight gain. This is also supported by the fact that aspartate aminotransferase (AST) which is an indicator of liver function and its metabolic activity was significantly lower (p<0.00) among birds that received 0.00 % dietary salt levels than those that had 0.25 % and 0.75 % salt levels. It is also possible that these enzymes were synthesized in response to the need to control osmotic balance due to higher metabolic rates triggered by higher dietary salt levels.

Conclusion

This present study showed that increasing dietary salt levels up to 0.75% resulted in better growth performance, increased live weight gain and hematological composition of broilers.

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