

Biochemical Blood Parameters of Broiler Chickens Fed Rice Milling Waste Based Diets

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Abstract

This experiment was conducted to evaluate the anti-nutritional factors, hematological and serum parameters of broiler chickens fed Rice Milling Waste (RMW) as a replacement for maize. The anti-nutritional factors were determined, also eleven experimental diets each were formulated to contain RMW as replacement for maize at 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. Around 307 old chicks were allocated to the 11 dietary treatments each having 3 replicate pens with 10 birds per pen in a Completely Randomized Design (CRD). Feed and water were provided ad libitum for 6 weeks. Data were collected on the hematology and serum biochemistry of broiler chickens with emphasis on serum enzymes which were analyzed using ANOVA with a ($p < 0.05$) level of significance. Result from anti-nutritional factors showed that rice milling waste were suitable as feed ingredient and as well would not hamper physiological response, hinder nutrient utilization and absorption of RMW and other ingredients present in the diet. The hematological indices and serum profile showed that the values were within the normal physiological range except for Packed Cell Volume (PCV) of broiler chickens fed 100% RMW (47.83%) which had a value above the normal range, a condition known as polycythemia, a disease state in which PCV is elevated which might be due to an increase in the number of RBC or to a decrease in the volume of plasma. Thus, the inclusion of RMW in diet of broiler chickens as a replacement for maize improved nutrient utilization and economic value. It can therefore be recommended that RMW can be used to replace maize between 10%-40% in the diets of broiler chickens.

Keywords: Rice milling waste; Anti-nutritional factors; Hematology; Serum; Broiler chickens

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Introduction

In developing countries, demand for poultry products had continued to increase. Report has shown that feed supply remains a major constrain in animal production due to the ever-increasing cost of conventional feed ingredients especially the energy and protein feed ingredients like maize, soybean cake and groundnut cake [1]. Maize which is a major ingredient in the poultry diets and its availability and price are influenced by competition between man, industry and livestock. According to a recent outlook report by Novus Agro Nigeria, findings revealed that there was over 83% price increase, as a metric ton of maize now sells for N183, 130 in Lagos, as against N100, 1000 sold last year [2]. With these trends, researchers/ farmers have continued to search for reliable and cheap alternatives on how to reduce cost of production of feed

despite increase in cost of ingredients.

Most of the alternative feed ingredients for maize contain Non-Starch Polysaccharide (NSP) [3]. These are forms of carbohydrates found in feedstuffs such as wholegrain cereals which is not digested in the small intestine and so provides no source of calories. However, the broilers digestive enzyme profiles are

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not designed to digest NSP, thereby limiting the broilers ability to utilize high fibre feedstuffs. This intolerable high fibre content causes digestive inefficiency of the gastro-intestinal tract thereby reducing the effect of digestive enzymes and absorption of nutrients [4]. The use of rice milling waste as an ingredient in animal feed, especially ruminants and poultry has been well documented [5]. Rice milling waste which is also known as rice offal or rice milling residue is a common by-product of rice milling that has over 36% crude fibre, 1200 kcal/kg, Metabolizable energy and 3.5% crude protein [6]. Birds are known to require a minimal amount of fibre in the diet for proper functioning of the digestive organs. Dietary fibre has been observed as a diluent of the diet and often an anti-nutritional factor. Nonetheless, moderate amount of fiber has the ability to improve the development of organs, enzyme production, and nutrient digestibility in poultry. Hematology and serum biochemistry assay of livestock suggests the physiological disposition of the animals to their nutrition and it has been found that various feed stuffs including unconventional sources may have effect on the hematology and serum biochemistry of livestock and hence concluded that feed ingredients may affect animals [7]. This study was therefore designed to determine the anti-nutritional factors of rice milling waste and also the effect of rice milling waste based diets on the hematology and serum biochemistry of broiler chickens.

Materials and Methods

Experimental site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture,

Umudike, Abia State, Nigeria. The area falls within the tropical rain forest zone, with an annual average rainfall of 2177 mm, temperature range between 20 °C-30 °C, with relative humidity of 50-59%, depending on season [8].

Determination of anti-nutritional factors

The following anti-nutrients were determined with the use of prescribed methods. Tannin was determined using A.O.A.C methods.

Experimental birds and management

A total of 330 day-old Arbor acre broiler chicks were procured for this study. The chicks were brooded together with a 60 W bulb in a brooding pen for the first seven days, thereafter; they were randomly divided into 33 groups of 10 birds each. Each group was raised in floor pens with wood shavings as litter material and contained feeders and drinkers for the provision of ad libitum access to feed and water respectively for duration of six weeks. Birds were vaccinated against Gumboro disease at 7th and 18th days of life while Newcastle disease was vaccinated at 12th day. Coccidiostats was administered to the birds during the experiment.

Experimental diets

Eleven experimental straight broiler diets were formulated such that the control diet did not contain rice milling waste, while substituting the dietary maize composition at the following levels of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. The composition of the diets and calculated analysis are shown in **Table 1**.

Table 1: Ingredients composition of straight diets without enzyme.

Ingredients	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G	Diet H	Diet I	Diet J	Diet K
Maize	56.3	50.67	45.04	39.41	33.78	28.15	22.52	16.89	11.26	5.63	-
RMW	-	5.63	11.26	16.89	22.52	28.15	33.78	39.41	45.04	50.67	56.3
SBM	26	26	26	26	26	26	26	26	26	26	26
FFS	10	10	10	10	10	10	10	10	10	10	10
Fish meal	4	4	4	4	4	4	4	4	4	4	4
Bone meal	3	3	3	3	3	3	3	3	3	3	3
Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100	100	100	100	100	100	100	100
Calculated composition											
Crude protein %	22.46	22.18	21.9	21.62	21.34	21.06	20.78	20.49	20.21	19.93	19.65
ME Kcal/kg	2923.6	2811	2698.4	2585.8	2473.2	2360.6	2248	2135.4	2022.8	1910.2	1797.6
Crude fiber %	4.05	5.42	6.82	8.23	9.64	11.05	12.95	13.86	15.26	16.68	18.08
Calcium %	1.45	1.45	1.45	1.45	1.45	1.45	1.37	1.45	1.44	1.44	1.44
Analyzed composition											
Dry matter %	88.48	88.94	88.68	89.18	89.23	89.28	89.41	89.53	89.59	89.76	89.96

Crude protein %	23.48	23.02	22.31	22.02	21.76	21.44	20.05	19.88	19.43	19.02	18.95
Ether extract	3.93	2.48	2.42	2.89	3.35	4.02	4.25	4.52	4.63	4.77	5.26
Crude fiber %	3.63	4.56	5.31	7.1	9.55	12.74	14.15	16.01	18.48	19.97	21.58
NFE %	56.9	58.82	58.59	55.7	50.5	47.48	46.52	43.77	40.1	39.02	36.64
ME Kcal/kg	2801.3	2713.89	2653.55	2628.01	2466.9	2401.22	2386.45	2283.4	2214.8	2198.23	2148.7

RMW: Rice Milling Waste; SBM: Soya Bean Meal; FFS: Full Fat Soya; NFE: Nitrogen Free Extract; ME: Metabolizable Energy; *Each 2.5 kg vitamin-mineral premix provided the following: A=8,000,000 iu, D3=2,000,000 iu, E= 5000 mg, K3=2000 mg, Folic acid=500 mg, Niacin=15,000mg, Calpan=5,000 mg, B2=8000 mg, B12=10,000 mg, B1=1,500 mg, B6=1,500 mg, Biotin=20 mg.

Hematological and serum profile

Blood samples (10 ml) were taken from the jugular vein at day-old and at the end of the experiment with a sterile needle, where 5 ml were kept in sample bottles containing Ethylene Diamine Tetraacetic Acid (EDTA), an anti-coagulant to prevent blood clotting. The blood samples were taken to the laboratory for hematological assessment such as Packed Cell Volume (PCV), Red Blood Cell (erythrocyte) count (RBC), Hemoglobin (Hb), White Blood Cell (leukocyte) count (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). The remaining 5 mls were kept in sample bottles without coagulant. Serum would be isolated by centrifugation at 2,000 rpm for 10 minutes and then stored at -70 °C until analysis. The serum constituents analyses includes total protein, glucose, albumin, globulin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP).

Data analysis

The experiments were laid out in a Completely Randomized Design (CRD). All data analyses were done using IBM® SPSS version 20.0. The data were then subjected to Analysis of Variance (ANOVA). Where treatment means are significant, separation of means were done using the Duncan's Multiple Range Test at 5% level of significance.

Completely randomized design model: $Y_{ij} = \mu + T_i + \epsilon_{ij}$

Where Y_{ij} = Individual observation

μ = Overall mean

T_i = Treatment effect

ϵ_{ij} = Random error.

Results and Discussion

Anti-nutritional factors of rice milling waste

The anti-nutrients value of rice milling waste is showed in **Table 2**. Anti-nutrient factors are substances present in the feed material that can inhibit or reduce their utilization if otherwise not properly removed or reduced to an innocuous level.

Table 2: Anti-nutritional factors of rice milling waste.

Parameters	Composition (%)
Phytate	0.57
Oxalate	0.39

Alkaloids	0.44
Flavonoids	0.01
Phenol	0.13
Steroids	0.01
Tannin	0.02
Saponin	0.33

Phytic acid is a unique natural substance found in plant seeds. It has received considerable attention due to its effects on mineral absorption. Phytic acid impairs the absorption of iron, zinc and calcium and may promote mineral deficiencies. Growth retardation in animals fed on rice bran diets was attributed to phytic acid interference with zinc metabolism and its present in RMW at a concentration of 0.57% [9]. Oxalates which are present in many plants and in significant amounts particularly in leafy greens, vegetables, fruits, cocoa, nuts and seeds. It binds to calcium and prevents its absorption in the body. It is present at a level of 0.39%. Alkaloids which are known to be basic, and have bitter taste [10]. The alkaloid content of RMW is 0.44%. Alkaloids have been found to be very effective against pathogenic microorganism. It was found in this present study that phenol is present in RMW at a concentration of 0.13%, while steroids were found at 0.01%. Tannin is an important secondary metabolite which reduces risk of coronary heart diseases [11]. They are water soluble phenolic compound, and have ability to precipitate proteins from aqueous solution. Tannin was found to be present in RMW at a concentration of 0.02%, while Saponins which are made of a steroid (or triterpene) group attached to a sugar moiety. These surface active compounds are found in legumes as well as certain spices and herbs. Saponins are able to lyse erythrocytes due to their interaction with cholesterol in the erythrocyte membrane [12,13]. It is present in RMW at a concentration of 0.33%. The results of the anti-nutritional factors showed that RMW would not hamper nutrient utilization and absorption by broiler chickens. Thus, broiler chickens can relatively handle the concentration of anti-nutritional factors present in RMW for optimum performance.

Blood profile of broiler chickens fed diet containing rice milling waste

The hematological indices of broiler chickens fed diet containing varying levels of Rice Milling Waste (RMW) shown in **Table 3**. The result of the Red Blood Cell (RBC) showed that broiler chickens fed diet containing 100% RMW were significantly higher ($p < 0.05$) than the remaining treatment groups except those fed 90% RMW, while the Packed Cell Volume (PCV) showed that broiler chickens

fed diet containing 100% RMW was significantly higher ($p < 0.05$) than the remaining treatment groups except those fed diet containing 70% and 90% RMW. The result of the Hemoglobin (Hb) showed that broiler chickens fed diet containing 100% were significantly higher ($p < 0.05$) than the remaining treatment groups but they were not significantly different ($p > 0.05$) from those fed 70% RMW, while the result of the White Blood Cell (WBC) showed that broiler chickens fed diet containing 100% RMW were significantly higher ($p < 0.05$) than the remaining treatment groups except those fed diet containing 30%, 40%, 50%, 70% and 80% RMW respectively. The result of the platelets showed that broiler chickens fed diet containing 100% RMW were significantly higher ($p < 0.05$) than those fed control diet, 20%, 30%, 60% and 80% RMW but they were not significantly different ($p > 0.05$) from the remaining treatment groups, while the result of the

Mean Corpuscular Volume (MCV) showed that broiler chickens fed diet containing 80% RMW were not significantly different ($p > 0.05$) from those fed control diet, 40% and 60% RMW but they were significantly higher ($p < 0.05$) than the remaining treatment groups. The result of the Mean Cell Hemoglobin (MCH) showed that broiler chickens fed diet containing 80% RMW were significantly higher ($p < 0.05$) than those fed diet containing 20%, 30%, 40%, 50%, 70%, 90% and 100% respectively but they were not significantly different ($p > 0.05$) from the remaining treatment groups, while the result of the Mean Corpuscular Hemoglobin Concentration (MCHC) showed that broiler chickens fed diet containing 100% RMW were significantly higher ($p < 0.05$) than the remaining treatment groups except those fed control diet, 10%, 30% and 70% RMW respectively.

Table 3: Haematological indices of broiler chickens fed diets containing varying levels of rice milling waste.

Parameters	T1 (control)	T2 (10%)	T3 (20%)	T4 (30%)	T5 (40%)	T6 (40%)	T7 (60%)	T8 (70%)	T9 (80%)	T10 (90%)	T11 (100%)	S.E.M
RBCx10 ¹² /L	2.70 ^{def}	2.61 ^{ef}	2.75 ^{cde}	2.85 ^{b^{cde}}	2.81 ^{b^{cde}}	2.97 ^{bcd}	2.62 ^{ef}	3.03 ^{bc}	2.40 ^f	3.10 ^{ab}	3.34 ^a	0.05
PCV (%)	41.80 ^{cdef}	39.70 ^{ef}	40.93 ^{def}	42.43 ^{b^{cde}}	43.37 ^{bcd}	43.73 ^{bcd}	40.50 ^{def}	45.50 ^{ab}	38.63 ^f	44.80 ^{abc}	47.83 ^a	0.53
Hb g/dl	10.37 ^{bc}	9.97 ^c	9.90 ^c	10.63 ^{bc}	10.65 ^{bc}	10.63 ^{bc}	10.07 ^c	11.50 ^{ab}	9.53 ^c	10.70 ^{bc}	12.47 ^a	0.17
WBCx10 ⁶ /L	174.07 ^c	179.80 ^{bc}	183.00 ^{bc}	193.57 ^{ab}	192.00 ^{ab}	183.67 ^{ab}	181.33 ^{bc}	189.00 ^{ab}	185.67 ^{abc}	174.33 ^c	198.33 ^a	1.68
Plateletsx10 ⁹ /L	42.67 ^{bcd}	54.33 ^{ab}	40.33 ^{bcd}	41.07 ^{bcd}	52.67 ^{ab}	50.00 ^{abc}	35.00 ^d	47.67 ^{abcd}	37.00 ^{cd}	54.00 ^{ab}	61.00 ^a	1.76
MCV fl	154.85 ^{ab}	152.30 ^b	149.08 ^{bc}	149.54 ^{bc}	154.29 ^{ab}	147.36 ^{bc}	154.90 ^{ab}	150.15 ^{bc}	160.97 ^a	144.61 ^c	143.08 ^c	1.04
MCH pg	38.40 ^{ab}	38.23 ^{ab}	36.05 ^{cd}	37.39 ^{bc}	37.85 ^b	35.82 ^{cd}	38.48 ^{ab}	37.87 ^b	39.72 ^a	34.54 ^d	37.29 ^{bc}	0.28
MCHC g/l	24.80 ^{bc}	25.10 ^{ab}	24.18 ^{bc}	25.02 ^{abc}	24.54 ^{bc}	24.31 ^{bc}	24.85 ^{bc}	25.23 ^{ab}	24.68 ^{bc}	23.88 ^c	26.06 ^a	0.13

a,b,c Means across rows with different superscripts differ significantly at $p < 0.05$; S.E.M: Standard Error of the Mean; RBC: Red Blood Cell; PCV: Packed Cell Volume; Hb: Hemoglobin; WBC: White Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

Hematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in hematological parameters have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on any living creature. For example, leucocytes are known to increase sharply when infection occurs, as they are one of the first lines of defense of the body [14].

The values obtained for the Red Blood Cell (RBC), Hemoglobin (Hb), White Blood Cell (WBC), platelets, MCV, MCH and MCHC are within the normal range for broiler chickens [15,16]. The values obtained in this study indicated no detrimental impact of RMW in diet of broiler chickens. The RBC, which is the total red blood count of the broilers in the present experiment did not present a definite trend to attribute the difference to dietary effect hence the MCH which is expression of the average hemoglobin content of a single RBC and the MCHC which is the expression of the volume within the RBC occupied by the hemoglobin [17]. The Hb which is the oxygen carrying protein matrix in the RBC was similar among all the groups which are an indication that RMW may have impacted on the Hb in the same way [18]. Although, the WBC of the broilers fed 100% RMW had a higher value than others. The primary role of the WBC is defense; it rises in event of issues it must fight. The Packed Cell Volume (PCV) of broiler chickens

fed 100% RMW (47.83%) had a value above the normal range, a condition known as polycythemia, a disease state in which PCV is elevated which might be due to an increase in the number of RBC or to a decrease in the volume of plasma.

The serum profile of broiler chickens fed diet containing varying levels of rice milling waste (RMW). The result of the total protein showed that broiler chickens fed 40% RMW was significantly higher ($p < 0.05$) than the remaining treatment groups, while the result of the glucose showed that broiler chickens fed control diet were significantly lower ($p < 0.05$) than the remaining treatment groups which were not significantly different ($p > 0.05$) from each other. The result of the Alanine Transaminase (ALT) showed that broiler chickens fed diet containing 90% RMW were significantly higher ($p < 0.05$) than remaining treatment groups except those fed 10%, 30%, 40%, 60%, 70%, 80% and 100% RMW, while the result of the Aspartate Transaminase (AST) showed that broiler chickens fed diet containing 100% RMW were significantly higher ($p < 0.05$) than those fed control diet, 10%, 20%, 40% and 50% RMW but they were not significantly different ($p > 0.05$) from the remaining treatment groups. The result of the Alkaline Phosphatase (ALP) showed that there were no significant differences ($p > 0.05$) across the treatment groups, while the Albumin showed that broiler chickens fed 30% and 40% RMW were not significantly different ($p > 0.05$) from each other but they were significantly higher ($p < 0.05$) than the remaining treatment groups is shown in **Table 4**.

Table 4: Serum profile of broiler chickens fed diets containing varying levels of rice milling waste.

Parameters	T1 (contro)	T2 (10%)	T3 (20%)	T4 (30%)	T5 (40%)	T6 (50%)	T7 (60%)	T8 (70%)	T9 (80%)	T10 (90%)	T11 (100%)	S.E.M
Total Protein g/dl	3.45 ^{cde}	3.57 ^{cde}	3.47 ^{cde}	4.23 ^b	4.84 ^a	3.15 ^{de}	2.99 ^e	3.29 ^{cde}	3.63 ^{cd}	3.50 ^{cde}	3.84 ^{bc}	0.1
Glucose mg/dl	111.00 ^b	188.65 ^a	184.33 ^a	177.67 ^a	235.33 ^a	188.33 ^a	206.33 ^a	211.67 ^a	210.33 ^a	212.00 ^a	197.67 ^a	7.21
ALT μ /l	16.00 ^{bc}	17.33 ^{abc}	14.00 ^c	16.67 ^{abc}	18.33 ^{abc}	16.33 ^{bc}	18.67 ^{ab}	18.00 ^{abc}	17.00 ^{abc}	21.00 ^a	19.00 ^{ab}	0.46
AST μ /l	19.67 ^b	20.33 ^b	18.67 ^b	21.33 ^{ab}	19.67 ^b	19.00 ^b	22.67 ^{ab}	22.33 ^{ab}	21.00 ^{ab}	21.67 ^{ab}	24.67 ^a	0.43
ALP μ /l	55.92	54.01	54.8	57.54	54.1	55.51	56.51	56.54	57.92	61.35	55.91	0.8
Albumin g/dl	2.03 ^b	1.89 ^{bc}	2.08 ^{bc}	2.93 ^a	2.88 ^a	1.74 ^{bc}	1.64 ^c	1.74 ^{bc}	2.06 ^{bc}	2.10 ^{bc}	2.22 ^b	0.08

a,b,c Means across rows with different superscripts differ significantly at p<0.05; S.E.M: Standard Error of the Mean; ALT: alanine transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase.

Serum biochemistry is a labile biochemical system which can reflect the condition of the organism and the changes happening to it under influence of internal and external factors. Total protein measures the total amount of protein present in the blood and specifically looks for the amount of albumin and globulin. The concentration of protein in blood is influenced by the nutritional state, hepatic function, renal function, occurrence of disease such as metabolic errors, that is, it is an indicator to detect unexplained weight loss, fatigue, edema, which is swelling caused by extra fluid in the tissues and to detect symptoms of kidney or liver disease. The result of the total protein ranged from 2.99-4.84 g/dl and thus suggest good quality protein of the test feedstuff since the higher the value of the total protein, the better the quality of the RMW [19,20]. Although the values obtained were higher than what was reported by Banerjee who suggested 2.00-3.5 g/dl but consistent with the range reported by Thrall and Clinical Diagnostic Division [21-23]. Also Marcos suggested that a higher total protein value indicates that the animal has less protein demands to tissues. While the glucose and albumin showed that there were no detrimental effect of RMW in the diet of broiler chickens except for albumin which showed that broiler chickens fed 50%, 60% and 70% RMW were lower than the normal range this might be due to the individual chicken selected for blood collection [24,25]. Although, Oladele reported total protein, albumin and glucose content of the blood of chickens are a factor of their health status and nutrient intake [26]. The author stated that adequately fed birds in good health are likely to have higher levels of blood proteins and as opposed to inadequately fed or under nourished birds. Also, lower blood glucose suggests that the birds might not be getting the required energy from their feed. The results of the albumin were within the range suggested that a higher albumin values showed higher ability as a clotting factor in preventing hemorrhage which were observed in broiler chickens fed RMW [27].

The liver enzyme, Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) are intercellular enzymes involved in amino acid or carbohydrate metabolism. These enzymes are present in high concentration in muscle, liver and brain. Elevations of concentrations of these enzymes in the blood indicate necrosis or disease, especially of

these tissues. The results were significantly affected by the varied inclusion of RMW which indicated normal physiological values, and values were within the recommended values [28-31].

Conclusion

The study examined the nutritional value of rice milling waste as possible replacement alternative for maize in the diets of broiler chickens and from the results obtained, the following conclusions were drawn:

- Rice milling waste was found to have minimal amount of anti nutritional factors which were observed to be acceptable without causing obstruction in nutrient digestibility and utilization.
- That the hematological indices and serum profile of broiler chickens fed RMW at varying levels were significantly affected however most of them were within the normal physiological range without having any deleterious effect on the birds.

It is hereby recommended that RMW can replace maize up to 40% without inclusion of exogenous enzymes to achieve optimum performance, economic gains and survivability of broiler chickens and enhanced product quality.

Conflict of Interest

None declared

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