



Evaluation of Fermented Corn Residue as a Growth Promoter in Broiler Chicken Diets

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Abstract	Article History
<p>Poultry production is one of the fastest-growing sectors in global agriculture, providing a major source of animal protein through meat and eggs. This study was undertaken to evaluate the effect of fermented corn chaff as a chicken additive for healthy broiler chicks. The effects of the fermented feeds on the broiler chicks were determined using <i>in vivo</i> techniques. The fermenter used in the study was identified to be <i>Lactobacillus acidophilus</i> strain DSN20079 (LADSM). The study revealed that there was a significant ($P < 0.05$) increase in the body weight of the test chicks fermented feed starting from week 1 (172 g) down to week 6 (1784 g). There was also an increase in the organ weights of the chicks (liver, kidney, lungs and heart). The feed conversion ratio (FCR) showed that in week 1, the FCR of the test group (2.471) was higher than the control group, and during weeks 2,3 and 4, the FCR of the control group was higher than the test group, but during weeks 5 and 6, the FCR of the test group was higher than the control group. The blood indices of the test chicks revealed that there was an increase in the counts of WBC, RBC, PLT, monocyte, basophil and lymphocyte but a reduction in the number of neutrophil and eosinophil. Therefore, the study concluded that the fermented corn chaff exhibited pronounced activity on the growth performance of the chicks and could be used as an additive for healthy broiler chicks.</p> <p>Keywords: <i>In vivo</i>, <i>Lactobacillus</i>, Chicks, Blood, Feed.</p>	<p>Received: 15 Aug 2025 Accepted: 22 Sept 2025 Published: 21 Sept 2025</p>  <p>Scan QR code to view*</p> <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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Introduction

Poultry production is a crucial component of global livestock farming, providing a significant source of animal protein through meat and eggs (FAO, 2021). Feed efficiency is a key determinant of profitability in broiler production, as feed costs account for up to 70% of total production expenses (Afolayan et al., 2019). The quest for cost-effective, nutritionally balanced, and environmentally sustainable feed additives has led to increased interest in agricultural by-products and fermentation technologies.

Corn chaff, a readily available by-product of maize processing, can be improved through fermentation, which enhances

digestibility, reduces anti-nutritional factors, and increases beneficial microbial populations (Adedokun et al., 2020). Fermentation also promotes the synthesis of bioactive compounds, such as organic acids, vitamins, and enzymes, that support gut health and immunity in poultry (Yang et al., 2022). When combined with essential micronutrients, fermented corn chaff can provide a more balanced nutrient profile, supporting optimal growth, bone development, and metabolic functions in broiler chicks (Hafeez et al., 2021).

The use of fermented agricultural by-products, such as corn chaff, aligns with sustainable livestock production goals by minimizing feed wastage, reducing environmental pollution,

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and supporting circular bioeconomy principles (Adeyemi and Olorunsanya, 2018). However, there is a need for further research on the specific effects of fermented corn chaff mixed with micronutrients on broiler chick growth performance, feed conversion ratio, and overall health. This study aims to address this knowledge gap by assessing the efficacy of fermented corn chaff with micronutrients as a feed additive in broiler chicken diets.

Materials and Methods

Isolation of the Test Sample

The media used for this isolation was de Man Rogosa and Sharpe broth (MRS) (BIOTECH). A 1.0 ml of fermented yoghurt (Aqua yoghurt) and banana extract were aseptically introduced into sterile Petri dishes (90 mm x 15 mm), then 20 ml of MRS which was prepared according to the manufacturers instruction and the procedures described in Cheesbrough (2010), Iheukwumere *et al.* (2020a), Iheukwumere and Iheukwumere, (2022a) and Iheukwumere *et al.* (2022b), was added into the plates, allowed to solidified. The plates were incubated in a microaerophilic environment (containing a candle used to evacuate all traces of oxygen, thereby creating an environment with only carbon dioxide). The incubation was done for 24 – 48 h at (30±2⁰C).

Purification of the Isolates

The plate that showed discrete colonies were selected after 24 - 48 h and each colony was aseptically streaked using a sterile wire loop on a sterile poured plate (90mm x 15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturers description, after which it was incubated at their required growth conditions (Iheukwumere and Iheukwumere, 2022c; Iheukwumere *et al.*, 2022d; and Iheukwumere and Iheukwumere, 2022e).

Characterization of the Bacteria Pure Isolates

The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2022f), Iheukwumere *et al.* (2023a) and Iheukwumere *et al.* (2023b).

Morphological characteristics of the Bacteria isolates

The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates were carried out as described in Iheukwumere *et al.* (2024) and Iheukwumere *et al.* (2022g). The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Obianom *et al.*, (2024), Egbe *et al.* (2025a) and Manasseh *et al.* (2025). The presence or absence of capsule was also carried out as described by Ekechukwu *et al.* (2025a). The presence or absence of flagellum was determined by carrying out motility test as described by Ekechukwu *et al.* (2025b).

Gram staining technique

A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolorizing the slide

content with 95%w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Ekechukwu *et al.* (2025c), Egbe *et al.* (2025b) and Egbe *et al.* (2025c).

Motility test: This was done using the method described by Iheukwumere *et al.* (2025a), Iheukwumere *et al.* (2025b) and Iheukwumere *et al.* (202c). A semi-solid medium prepared by mixing 5.0g of bacteriological agar (BIOTECH) with 2.0g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10 ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35±2⁰C for 24h.T.

Biochemical characteristics of the isolates

Indole test: Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. This was done using the method described by Iheukwumere *et al.* (2025d), Iheukwumere *et al.* (2025e) and Iheukwumere *et al.* (2025f). The isolates were cultured in peptone water in 500.0 ml of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37°C for 48 hr. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The capability of the isolates to metabolize some sugars (glucose, xylose, ducitol, maltose, arabinose, inositol, mucate and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution were prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes as described by Dim *et al.* (2025a) and Dim *et al.* (2025b). The medium was then inoculated with the appropriate isolates and the cultures incubated at 37°C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted.

Methyl red test: Using the method described by Dim *et al.* (2025c), Iheukwumere *et al.* (2025g). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized

medium. This was incubated at 37°C for 48 hr. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution was added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: Using the method described by Iheukwumere *et al.* (2025h), Ike *et al.* (2025a). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48hr. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α -naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37°C for 48 hr. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained as described by Ike *et al.* (2025b) and Ike *et al.* (2025c).

Catalase test: The test was carried out as described by Ike *et al.* (2025d) and Ike *et al.* (2025e). A smear of the isolate was made on a cleaned, grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H₂O₂) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out using the method described by Ugwu *et al.* (2025a). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Urease test: This was carried out as described by Ugwu *et al.* (2025b). The urea agar slant was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into sterilized medium. This was incubated at 37°C for 48 h. After incubation, observation was made for the presence of purple-pink colouration.

Molecular characterization of the isolates

Extraction and purification of DNA: All strains were plated on Nutrient Agar (Biotech) and incubated at 37°C for 24 hr. Using the Zymo Research (ZR) DNA miniprep™ kit (Category No. D6005; Irvine, California, USA), bacterial genomic DNA was extracted and purified as described by Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2020) with the procedures outlined in the kit.

Determination of the quality of extracted DNA: Using mass spectrophotometer (Nanodrop), One micro litre (1µL) was aseptically dropped into a fresh space in the chamber and the chamber was lightly closed which was then linked to a computer system which showed the window that discovered

the value of the sample at 260/280nm as described by (Iheukwumere *et al.*, 2017a; Chude *et al.*, 2020).

Amplification of DNA and gel electrophoresis of PCR product: This was analysed using Master cycler Nexus Gradient (Eppendorf). A mixture of primer (20 µL), template DNA (20µL), water (72 µL) and master mix (108 µL), which comprises taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride (MgCl₂) and nucleotides triphosphates (NdTPs), was made in 1.5 mL tube and homogenized using vortex mixer (Eppendorf). This was then positioned in the block chamber of the master cycler and then programmed. The PCR program for conditions were as follows: initial incubation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 15 secs, annealing at 55°C for 15 secs, elongation at 72°C for 21 secs and final extension period for 10 mins at 72°C. The amplified products were electrophoresed in 1.0% agarose gel and a1kb DNA ladder was used as a size reference. After staining with 3µL of nucleic acid stain (GR green), the gel was documented with gel documentation apparatus (Iheukwumere *et al.*, 2017b; Iheukwumere *et al.*, 2017c; and Iheukwumere *et al.*, 2018b).

DNA sequencing of 16s rRNA fragment:The 16S rRNA amplified PCR products generated from universal primer (16S), was used for the sequencing using ABI DNA sequencer (Applied Biosystem Inc) at International Institute of Tropical Agriculture (IITA), Ibadan using the method of Iheukwumere *et al.* (2017d), and Iheukwumere *et al.* (2018c).

Computational Analysis: This was analysed making use of the modified method of Iheukwumere *et al.* (2025i) and Iheukwumere *et al.* (2025j). The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned using pair wise alignment tool. The consensus sequences formed by the alignment of the forward and reverse sequences were used to perform the Basic Local Alignment Search Tool (BLAST) using National Centre for Biotechnology Information BLAST over the internet. The sequences of the isolates with 95% and above similarities were accepted. Also the maximum scores, total scores and accession numbers of the isolates were assessed. The relatedness of the isolates was determined by tracing their phylogenetic tree using DNA distance neighbour phylogenetic tree tool.

Preparation of Feed Supplement

Preparation of the corn chaff

The corn chaff was properly collected from the appropriate sites, washed and air dried. The material was ground using an electrical blender, packed in 500 ml beaker (PYREX) sealed with aluminium foil and then autoclave at 121°C for 15 min at 15psi.

Fermentation Process

This was carried out using the modified method of Iheukwumere *et al.* (2017), Iheukwumere *et al.* (2022) and Iheukwumere *et al.* (2025k). After autoclaving, a 100 g of the sterile sample was weighed into another 250 ml beaker (PYREX) using analytical weighing balance, which was properly sterilized using electric oven at 180°C for 2 h, This

was then inoculated with the fermenter (10 ml) prepared and diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.6mL of 1% BaCl₂. 2H₂O and 99.4 mL of 1% Conc. H₂SO₄. This was allowed for 7 days.

Storage and packaging

After fermentation, the fermented samples were aseptically dried using an electric oven at 80°C for 7days. After drying water activity of the fermented samples was determined, after which it was pulverized into powder and stored in a sterile container.

Moisture Content Determination

A crucible was dried, cooled, and weighed (initial weight recorded as W₁). Then, 2.0 grams of the sample was added to the crucible, and its weight was recorded as W₂. The crucible with the sample was heated in an oven at 105°C for 4 to 6 hours. After heating, the final weight of the crucible and its contents was measured (final weight recorded as W₃). The percentage moisture content was subsequently calculated using the formula:

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Experimented Chicks: A total of twenty four (24) broiler chicks (3 weeks old) were purchased from poultry market located at Ihiala market, Ihiala L. G. A. in Anambra State were used for the study. The chicks were kept in separate, thoroughly cleaned and disinfected house and provided with feeds and water ad libitum. All the chicks were vaccinated against Newcastle disease using Lasota vaccine strains at 6 and 19 days of age, against infectious bronchitis using live H120 strain at 6 days old and also against avian influenza (A1) disease using inactivated H5N1 virus vaccine strain at 7 days old. All the vaccines were given via eye drop instillation except (A1) vaccine, which was given through the subcutaneous route at the back of the neck from the folder report collected from the poultry farmer.

Feed Additive

The fermented corn chaff was mixed with micronutrients and chicken feed in a 1:20 ratio. This mixture was thoroughly mixed and administered to the chicks. The chicks were divided into two groups (A and B). Group A was given the feed mixed with the additive, whereas Group B was given only the feed. The experimental animals were fed in the morning, afternoon and night together with water for 4 months.

Experimental Protocols for the *In vivo* Models: A total of 36 broiler chicks were used for this study. The broiler chicks were grouped into six groups, and each group comprises 6 chicks. A 0.5 mL/100 g of corn chaff was orally administered to each of the groups of broiler chicks, and the remaining group was given only feed and water as a control group. The body weights and blood absolute lymphocytes were assessed from the blood samples drawn from the chicks after 11 days.

Body weights: The body weights of the experimented rats were checked and recorded weekly using electronic weighing balance (LXD200) and recorded as described in the work

published by Ejike *et al.* (2017), Nwobodo *et al.* (2018) and Ekésiobi *et al.* (2025).

Hematological Indices: The blood samples collected from the broiler chicks were examined using Automated Hematology Analyzer (MIN DRAY BC – 360), and the variations in the red blood cells (RBCs), lymphocytes, monocytes, neutrophils, eosinophils and basophils were assessed and recorded as described in the work published by Agiang *et al.* (2017)

Statistical Analysis: The data obtained in this study were presented in tables and figures. Their percentages were also calculated. The sample means and standard deviations of some of the analytical data were also calculated. The significance of this study was determined at 95% using one way analysis of variance (ANOVA). Pairwise comparison was analyzed using student “t” test as described by Okeke *et al.* (2017), Iheukwumere *et al.* (2014), Iheukwumere *et al.* (2017e), Nwike *et al.* (2017), Amadi *et al.* (2017), and Iheukwumere *et al.* (2025).

Results

The cultural and morphological characteristics of the fermenter are shown in Table 1. The result revealed that isolate P showed cream white appearance on MRS agar with a low convex elevation, smooth edge, and surface. The isolate has a Gram-positive rod, non-sporing and non-motile rods. The biochemical characteristics of the fermenters revealed that isolate P was catalase, citrate, oxidase, urase, gelatin, methyl red, Voges Proskauer negative. The isolate showed complex utilization to glucose, lactose, maltose and fructose while it was unable to utilize xylose and sorbitol. The isolate showed varying utilization to D mannitol, inositol, Trehalose and Dulcitol as shown in table 2. The nucleic acid extracted from the fermenters revealed that it was deoxyribonucleic acid (DNA) at the ratio of 260nm/280nm ranging from 1.80-1.83 as shown in Table 3. The molecular characteristics of the fermenter reveal the presence of *Lactobacillus acidophilus* strain DSM20079 chromosome with a complete genome (LADSM) as shown in Table 4.

The body weight of the chicks when feed with fermented corn chaff revealed that there was a significant (P<0.02) increase in the body weight of the chicks compared to the control group and this was very pronounced at week six of feeding the chicks with the fermented feed supplement as shown in Table 5.

The result of the organ weight of the chicks after been feed with fermented supplement (corn chaff) revealed that there was an increase in the organ weights (Liver, Kidney, Lungs, Heart) of the test chicks compared to the control group and these changes were statistically significant (P<0.05) as shown in Table 6.

The feed conversion ratio and feed intake of the chicks in shown in table 1. The result revealed that during the first week, that FCR of the test group (2.471) was higher than the control group (2.143), during the second week the FCR of the control group was higher than the test group (3.217), and also in week three the FCR of the control group was also higher than the test group same as week four but during week 5 and 6 the FCR of the test group was higher than the control group, also the result

revealed that weight and weight gain of the test group was higher than the control group and this indicates that the fermented feed supplement (corn chaff) had a significant effect on the broiler chicks as shown in Table 7.

The blood indices of the chicks fed with the fermented feed supplement (corn chaff) revealed that there was an increase in the counts of WBC, RBC, and in the percentage of monocyte, basophil and lymphocyte of the test group but reduced the counts of PLT and number of the Neutrophil, Eosinophil amongst the test group compared to the control group as shown in Table 8.

Table 1: Cultural and morphological characteristics of the fermenter

Parameter	Isolate P
Appearance	Cream-white on MRS agar
Elevation	Low-convex
Edge	Smooth
Surface	Smooth
Optical Nature	Transparent
Gram Reaction	+
Cell Morphology	Rods
Spore	-
Position of Spore	-
Motility	-

++ Positive; - = Negative

Table 2: Biochemical characteristics of the fermenters

Parameter	Isolate P
Catalase	-
Citrate	-
Oxidase	-
Urease	-
Gelatin	-
Methyl Red	-
Voges Proskauer	-
Glucose	+
D-mannitol	+/_
Lactose	+
Maltose	+
Xylose	-
Inositol	+/_
Fructose	+
Sorbitol	-
Trehalose	+/_
Dulcitol	+/_
Possible Isolate	<i>Lactobacillus</i> species

Table 3: Authentication of nucleic acids extracted from the fermenters

Sample ID	Nucleic Acid Conc(μ g/mL)	260 nm	280 nm	260/280
P	142.40	3.1915	1.7440	1.83

Table 4: Molecular identities of the fermenters

Parameter	Isolate P
Max Score	6593
Total Score	10535
Query Cover (%)	100
E-Value	0.0
Identity (%)	100
Accession Length	2009973
Accession Number	CP020620.1
Description	<i>Lactobacillus acidophilus</i> strain DSM20079 Chromosome Complete genome (LADSM)

Table 5: Body weights of the chicks

Week	Control Group	Test Group
1	168	172
2	376	394
3	660	693
4	974	994
5	1220	1381
6	1442	1784

Table 6: Organ weight of the chicks

Organ	Control group	Test group
Liver (g)	7.40 \pm 0.01	7.42 \pm 0.01
Kidney (g)	0.52 \pm 0.01	0.54 \pm 0.01
Lungs (g)	1.31 \pm 0.01	1.34 \pm 0.01
Heart (g)	0.67 \pm 0.01	0.69 \pm 0.01

Table 7: Feed intake and feed conversion ratio among the chicks

Week	Control Group				Test Group			
	Feed (g)	Weight (g)	Weight gain (g)	FCR	Feed (g)	Weight (g)	Weight gain (g)	FCR
1	28	168	60	2.143	34	172	84	2.471
2	62	376	208	3.355	69	394	222	3.217
3	102	660	284	2.784	117	693	299	2.556
4	146	947	287	1.966	158	994	301	1.905
5	194	1220	273	1.407	208	1381	387	1.861
6	243	1442	222	0.914	268	1784	403	1.504

Table 8: Hematological indices (Corn chaff)

Parameter	Control Group	Test Group
WBC (X10 ⁹ L)	12.88	13.18
RBC (X10 ¹² L)	7.37	7.52
PLT (X10 ⁹ L)	825.00	822.00
Neu (%)	40.60	28.70
Eos (%)	4.75	1.15
Mon (%)	3.85	5.25
Bas (%)	0.10	0.10
Lym (%)	50.70	64.80

Discussion

Fermented feeds have been shown to reduce anti-nutritional factors, improve palatability, increase beneficial microflora in the gut, and enhance the bioavailability of nutrients and when applied to corn, fermentation can improve its amino acid profile, reduce mycotoxin risks, and increase lactic acid production, which benefits gut health and pathogen resistance in poultry (Sundaram *et al.*, 2021). This present study focuses on the assessment of fermented corn chaff as a chicken additive for healthy broiler chicks. The cultural, morphological and biochemical characteristics of the *Lactobacillus* species agree with the findings of many researchers (Tufail *et al.*, 2011; Mannan *et al.*, 2017; Divisekera *et al.*, 2019; and Celik *et al.*, 2021) who isolated and characterized *Lactobacillus* species from yoghurt and banana. The ability of the isolates to utilize common sugars such as glucose, lactose, sucrose, maltose and some alcoholic sugars such as mannitol, sorbitol and xylitol indicates their potential to produce beneficial metabolites which support gut health and aid food fermentation. These potentials were also described by many researchers (Tufail *et al.*, 2011; Mannan *et al.*, 2017; Divisekera *et al.*, 2019; and Celik *et al.*, 2021). The molecular characterization of the *Lactobacillus* species revealed the presence of *Lactobacillus acidophilus* strain DSM20079 (LADSM). The occurrence of *Lactobacillus acidophilus* in strains had been reported by Li *et al.* (2011), Anjum *et al.* (2014), and Mannan *et al.* (2017), who stated that the occurrence may be due to antagonism.

The result of the fermented corn chaff meal which was fed to broiler chicks, indicated an increase in weight gain, body weight, organ weight and immune response. This finding corroborated with the findings of by many researchers (Baluogu *et al.*, 2017; Onunkwo and Ekine, 2020; Yang *et al.*, 2020), who evaluated the effect of fermented chicken feather on broiler chicks.

Conclusion

This study has shown that fermented corn chaff exhibited pronounced activity on the growth performance of the chicks and could be used as an additive for healthy broiler chicks.

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