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Full Length Research Paper

Physico-chemical analysis and nutrient retention of mixed-culture fungal fermented mango (*Mangifera indica*) kernel cake in cockerels

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The study was conducted to evaluate the efficacy of mixed-culture fungal fermented mango (*Mangifera indica*) kernel cake (MKC) as chicken feed ingredient. Four fungi namely *Rhizopus oligosporus* (Ro), *Aspergillus niger* (An), *Rhizopus stolonifer* (Rs) and *Penicillium chrysogenum* (Pc) were mixed as starter inoculums to ferment MKC. Eight treatments consisting of RoAn fermented MKC (T1), RoRs fermented MKC (T2), RoPc fermented MKC (T3), AnRs fermented MKC (T4), AnPc fermented MKC (T5), RsPc fermented MKC (T6), unfermented MKC (T7) and maize as control ingredient (T8) were used for nutrient retention trial. One hundred and eight cockerels were allotted for the treatments ingredients for 72 h using a completely randomized design. The birds were fed on the ingredients with a tubular instrument, while water was administered *ad-libitum* during the experimental period. The pH (3.50) of RoRs fermented MKC decreased significantly ($p < 0.05$) compared with unfermented MKC which had highest pH 5.13 at 168 h. Majority of the fermented MKC had higher ($p < 0.05$) titratable acidity (8.25 - 15.37 mg/100 g) compared with unfermented MKC (10.61 mg/100 g). Between 5.62 and 11.05% of MKC biomass was lost during fermentation which was higher ($p < 0.05$) at 168 h compared with unfermented MKC. Crude protein of the fermented MKC was significantly ($p < 0.05$) higher than the values of unfermented MKC, while crude protein of RsPc fermented MKC (21.93%) and RoRs (23.96%) fermented MKC were higher ($p < 0.05$) than other treatments. A great extent of the mixed-culture fermented MKC had higher ($p < 0.05$) total carbohydrate and glucose compared with unfermented MKC. Between 59 and 80 min were obtained as transit time for birds fed on the mixed-culture fungal fermented MKC. Most of the values were higher ($p < 0.05$) than 62 min obtained for the unfermented MKC (T7). Birds fed on RoAn fermented MKC (T1) had highest feed intake (28.34 g/day/bird), while those fed on RoRs fermented MKC (T2) had lowest feed intake (21.90 g/day/bird) but had higher ($p < 0.05$) nutrient retention (59.45%). The values of average nutrient retention of the mixed-culture fermented MKC were significantly ($p < 0.05$) higher than the values of unfermented MKC (39.72%), except for RsPc fermented MKC (T6) that had 38.56%. Ranking according to average nutrient retention (ANR) of the birds fed on the test ingredients is as follows: RoRs (T2) > RoPc (T3) > Maize (T8) > RoAn (T1) > AnPc (T5) > AnRs (T4) > RsPc (T6). The study indicates that mixed-culture fungal fermented MKC had significant ($p > 0.05$) increase in titratable acidity, crude protein, glucose and biomass loss with decreased pH. There was improvement in transit time and significant ($p > 0.05$) increase in nutrient retention of the fermented MKC compared with unfermented MKC. In conclusion, results of the fermented MKC were comparable with maize which makes it suitable as a potential feed ingredient in chicken's diet.

Key words: Unfermented mango kernel cake, fermented mango kernel cake, mixed-culture starter inoculum, transit time, nutrient retention.

INTRODUCTION

Mixed-culture fermentations are those in which the inoculum's always consists of two or more organisms. The cultures can consist of known species to the exclusion of all others, or they may be composed of mixtures of unknown species. Mixed-cultures may be of one microbial group that is, consists of only bacteria or fungi, and sometimes they may consist of a mixture of fungi and bacteria or fungi and yeasts or other combinations in which the components are quite unrelated (Carpenter, 1972 and Jay,1987). All of these combinations are encountered in oriental food fermentations (Hesseltine, 1983). One example of complex sequential interaction of two fermen-tations which employ fungi, yeast and bacteria is the manufacture of *miso*. This oriental food fermentation product is produced by the fermentation of soybeans, rice and salt using the mixed-culture of *Aspergillus oryzae* and *Aspergillus soyae* to make a paste-like fermented food.

Miso is used as a flavouring agent and as a base for *miso* soup (Hesseltine, 1983). Hesseltine (1983) reported some advantages of mixed-culture over mono-culture fermentation which includes: Probability of higher product yield, production of essential growth factors or compounds that are beneficial to a second microorganism, alteration in pH that may improve the activity of one or more enzymes, elevated temperature that may promote growth of second microbe, occurrence of a remarkably stable association of microorganisms, production of compounds such as alcohol and organic acids that could work to the exclusion of unwanted microorganisms and better utilization of fermented substrate. Harrison (1978) succinctly summarized in his future prospects of mixed-culture fermentations that no claim for novelty can be made for mixed-cultures since they form the basis of most ancient fermentation processes, although, with the exploitation of mono-cultures having been pushed to its limits. It is perhaps time to reappraise the potential of mixed-culture systems in the fermentation of unconventional tropical seeds which can then be used as animal feedstuff. This study was therefore designed to assess the physicochemical properties and nutrient retention of the mixed-culture fungal fermented "Oori" mango (*Mangifera indica*) kernel in cockerels.

MATERIALS AND METHODS

Preparation of mixed-culture starters

The inoculums used for the study were RoAn: *Rhizopus oligosporus* (50 ml containing 5 x 10^4 spore/ml suspension) mixed with *Aspergillus niger* (50 ml containing 5 x 10^4 spore/ml suspension); RsPc: *Rhizopus stolonifer* (50 ml containing 5 x 10^4 spore/ml suspension) mixed with *Penicillium chrysogenum* (50 ml containing 5 x 10^4 spore/ml suspension); RoRs: *R. oligosporus* (50 ml containing 5 x 10^4 spore/ml suspension) mixed with *R. stolonifer* (50 ml containing 5 x 10^4 spore/ml suspension); AnPc: *A. niger* (50 ml containing 5 x10^4 spore/ml suspension) mixed with *P. chrysogenum* (50 ml containing 5 x 10^4 spore/ml suspension); AnRs: *A. niger* (50 ml containing 5 x 10^4 spore/ml suspension) mixed with *R. stolonifer* (50 ml containing 5 x 10^4 spore/ml suspension); RoPc: *R. oligosporus* (50 ml containing 5 x 10^4 spore/ml suspensions) mixed with *P. chrysogenum* (50 ml containing 5 x 10^4 spore/ml suspension).

Preparation and fermentation of "Oori" mango kernel cake (MKC)

MKC was prepared as described by Kayode and Sani (2008). One-kilogramme of autoclaved (sterile) MKC was mixed with 1 litre of sterile distilled water in six (6) different fermenters and stirred properly to obtain a uniform mash as described by Kayode and Sani (2009). Twenty millilitres of the mixed-culture starter suspension (5 x 10^4spore/ml) was added and mixed before commencement of fermentation and incubated at ambient temperature (27 ± 5˚C) for 168 h (Lawal et al., 2005). Fermentation data were recorded daily for 7days.

Physico-chemical analysis of the mixed-culture fermented "Oori" mango kernel cake

The method of AOAC (2000) was used to determine titratable acidity and proximate analysis. Biomass was assessed according to Karunananda et al. (1992). Glucose fraction was analyzed colorimetrically using dinitro-salicylic acid (DNSA) method (Miller, 1959), while, pH was measure with a Crison micro pH meter (Model 2000).

In-vivo nutrient retention trials of the mixed-culture fermented "Oori" MKC using matured cockerels

Nutrient retention trial was conducted with sixteen (16) weeks old cockerels. Twelve feed ingredients were prepared, made of a maize feed as control, unfermented MKC, and six mixed-culture fermented mango kernel cakes. One hundred and eight cockerels were used for the 12 treatments. Each treatment was replicated thrice and each replicate contained 3-cockerels. One hundred and twenty grammes for a day of the experimental feed ingredients were fed and water supplied *ad-libitum.* Birds were fasted for 24 h before the commencement of feeding trial which lasted for 72 h. In the course of the experiment, data was recorded on feed transit time. Amount of feed consumed, the orts taken and total collection was made of the feaces voided. The feaces was oven dried at 60°C prior to analysis for fat, fibre, protein and carbohydrate retention (AOAC, 2000).

RESULTS AND DISCUSSION

There was steady decrease in pH of the MKC during fermentation. The pH (3.50) of the *R. oligosporus* and *R. stolonifer* mixed-culture fermented MKC decreased significantly ($p < 0.05$) compared with the unfermented MKC which had the highest pH 5.13 after 168 h (Table 1). The titratable acidity of the MKC during fermentation for 168 h is shown in Table 2. The values of titratable acidity of fermented MKC which ranged from 8.25 - 15.37 mg/100 g at 168 h were significantly ($p < 0.05$) different compared

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Abbreviations: MKC, Mango kernel cake; **Ro,** *Rhizopus oligosporus*; **An,** *Aspergillus niger*; **Rs,** *Rhizopus stolonifer*; **Pc,** *Penicillium chrysogenum*; **ANR,** average nutrient retention; **DNSA,** dinitro-salicylic acid.

Table 1: Effect of mixed-culture fermentation at ambient temperature (27±5^{0}C) on pH of “Oori” mango kernel cake.

Mixed-culture fermented MKC	Fermentation period (h)							
	0	24	48	72	96	120	144	168
R.oligosporus and *A. niger* (RoAn)	5.13 ^{a}	5.12^a	4.98^{ab}	4.83^{ab}	4.61^{bc}	4.15^b	4.08^c	4.07^c
R. stolonifer and *P. chrysogenum* (RsPc)	5.13^a	5.13^a	4.95^a	4.86^b	4.72^c	4.13^b	4.13^c	4.06^c
R.oligosporus and *R. stolonifer* (RoRs)	5.13^a	5.13^a	4.99^{ab}	4.66^a	4.42^b	4.02^a	3.58^a	3.50^a
A. niger and *P.chrysogenum* (AnPc)	5.13^a	5.12^a	4.99^{ab}	4.91^b	4.81^c	4.80^d	4.34^a	4.24^d
A. niger and *R. stolonifer* (AnRs)	5.13^a	5.13^a	5.01^{ab}	4.92^b	3.94^a	3.92^a	3.89^b	3.73^b
R.oligosporus and *P. chrysogenum* (RoPc)	5.13^a	5.13^a	4.97^a	4.74^{ab}	4.62^{bc}	4.50^c	4.52^e	4.50^e
Unfermented MKC	5.13^a	5.13^a	5.13^b	5.13^c	5.12^d	5.13^e	5.12^f	5.13^f
SEM	0.00	0.82	1.36	1.59	2.01	1.03	1.59	1.69

Values are means of three replicates readings; SEM = standard error of mean; means within column having different superscripts differ significantly ($p < 0.05$).

Table 2. Effect of mixed-culture fermentation at ambient temperature (27±5ºC) on titratable acidity of “Oori” mango kernel cake.

Mixed-culture fermented MKC	Fermentation period (h) /titratable acidity (mg/100g)							
	0	24	48	72	96	120	144	168
RoAn	10.61^a	10.80^a	11.27^{bc}	12.83^d	12.73^c	13.97^e	14.82^e	15.37^e
RsPc	10.61^a	10.85^a	12.10^d	12.13^c	12.21^c	11.85^c	10.61^b	8.25^a
RoRs	10.61^a	10.92^a	11.95^d	12.36^c	12.65^c	12.55^d	12.67^{cd}	12.80^c
AnPc	10.61^a	11.10^a	11.30^{bc}	11.06^b	11.10^{ab}	8.65^a	8.08^a	8.25^a
AnRs	10.61^a	11.17^a	11.68^{cd}	12.30^c	12.65^c	12.81^d	13.57^{de}	14.08^d
RoPc	10.61^a	10.80^a	11.08^b	11.45^b	11.38^b	11.64^c	11.84^{bc}	11.88^c
UMKC	10.61^a	10.61^a	10.61^a	10.61^a	10.61^a	10.61^b	10.61^b	10.61^b
SEM	0.00	1.71	1.39	1.27	2.11	2.11	1.39	0.28

Values are means of three replicates readings; SEM = Standard error of mean; means within column having different superscripts differ significantly ($p < 0.05$); *R. oligosporus* and *A. niger* (RoAn), *R. stolonifer* and *P. chrysogenum* (RsPc); *R. oligosporus* and *R. stolonifer* (RoRs), *A. niger and P. chrysogenum* (AnPc); *A. niger and R. stolonifer* (AnRs), *R. oligosporus and P. chrysogenum* (RoPc); unfermented mango kernel cake (UMKC).

with values (10.61 mg/100 g) of unfermented MKC. It is shown in Table 3 that between 5.62 and 11.05% of MKC, biomass was lost during mixed-culture fermentation after 168 h. The values of biomass loss of the mixed-culture fermented MKC were significantly ($p < 0.05$) higher at 168 h compared with unfermented MKC and their mono-culture counterparts as reported by Kayode et al. (2009).

There was slight variation in proximate composition of the mixed-culture fermented MKC (Table 4). The crude protein (18.05 - 23.96%) and glucose (17.82 -20.61 mg/g) of the mixed-culture fermented MKC were higher ($p < 0.05$) compared with crude protein (15.56%) and glucose (15.12mg/g) of the unfermented MKC. Total carbohydrate (56.34 - 63.60%), crude fibre (1.29 - 2.49%) and crude fat (4.05 - 6.20%) of the fermented MKC were lower ($p < 0.05$) than the values (64.84, 2.64 and 6.98%) of the unfermented MKC, respectively. There was similarity in the pattern of reducing sugar production of the fermented MKC. The values were significantly ($p < 0.05$) different among the treatments means right from 24-168 h of fermentation. However, the range of values of reducing sugar (15.12 -22.18 mg/g) of the mixed-culture fermented MKC (Table 4) was higher ($p < 0.05$) compared with the values (15.17 mg/g) of unfermented MKC. The transit time for birds fed on the mixed-culture fungal fermented MKC (T1 - T6) (Figure 1) was between 59 and 80 min. Most of the values were significantly ($p < 0.05$) higher than 62 min obtained for the unfermented MKC (T7). The birds fed on *R. oligosporus and A. niger* fermented MKC (T1) had highest feed intake (28.34 g/day/bird), while

Table 3. Effect of mixed-culture fungal fermentation at ambient temperature (27±5ºC) on the biomass of "Oori" mango kernel cake.

Mixed-culture fermented MKC	Fermentation period (h) / biomass (%)							
	0	24	48	72	96	120	144	168
RoAn	100[a]	99.07[d]	98.72[cd]	96.63[b]	96.32[b]	95.02[b]	93.87[ab]	93.55[cd]
RsPc	100[a]	98.08[c]	96.30[a]	96.42[b]	96.25[b]	95.86[b]	94.95[b]	94.38[b]
RoRs	100[a]	96.90[a]	95.87[a]	93.95[a]	93.85[a]	92.75[a]	93.40[ab]	92.46[bc]
AnPc	100[a]	99.87[d]	99.30[d]	98.96[c]	93.30[a]	92.11[a]	92.04[a]	91.92[b]
AnRs	100[a]	99.85[d]	97.60[bc]	95.83[b]	94.72[ab]	92.15[a]	92.10[a]	91.66[b]
RoPc	100[a]	97.40[b]	6.10[a]	96.05[b]	96.15[b]	93.53[a]	93.52[ab]	88.92[a]
UMKC	100[a]	99.99[d]	99.98[d]	99.97[c]	99.99[c]	99.96[c]	100[c]	99.99[e]
SEM	0.00	1.30	0.43	0.37	0.57	0.47	0.67	0.43

Values are means of three replicates determinations; SEM = standard error of mean; means within column having different superscripts differ significantly ($p < 0.05$); *R. oligosporus* and *A. niger* (RoAn); *R. stolonifer* and *P. chrysogenum* (RsPc); *R. oligosporus* and *R. stolonifer* (RoRs), *A. niger and P. chrysogenum* (AnPc); *A. niger and R. stolonifer* (AnRs), *R. oligosporus and P. chrysogenum* (RoPc); unfermented mango kernel cake (UMKC).

Table 4. Proximate composition of the mixed-culture fungal fermented "Oori" mango kernel cake.

Mixed-culture fermented MKC	Proximate composition (%)						
	Moisture content	Crude fat	Crude protein	Crude fibre	Total ash	NFE (CHO)	Glucose (mg/g)
RoAn	7.80[a]	4.05[a]	19.81[ab]	2.18[ab]	5.80[bc]	60.36[ab]	19.11[c]
RsPc	7.25[a]	5.23[ab]	21.93[ab]	1.29[a]	4.17[ab]	60.13[ab]	19.66[c]
RoRs	6.07[a]	6.20[ab]	23.96[b]	2.05[ab]	4.60[abc]	57.12[ab]	17.82[ab]
AnPc	6.90[a]	4.17[a]	19.89[ab]	1.90[a]	4.73[abc]	62.41[ab]	20.61[c]
AnRs	6.02[a]	5.01[ab]	18.05[ab]	2.13[ab]	5.19[bc]	63.60[ab]	18.79[ab]
RoPc	7.15[a]	4.93[ab]	22.48[b]	2.49[b]	6.61[c]	56.34[a]	19.35[c]
UMKC	7.35[a]	6.98[b]	15.56[a]	2.64[b]	2.63[a]	64.84[b]	15.12[a]
SEM	0.62	0.69	1.84	0.50	0.60	2.15	1.07

Values are means of three replicates determinations; SEM = standard error of mean; means within column having different superscripts differ significantly ($p<0.05$); *R. oligosporus* and *A.niger* (RoAn); *R. stolonifer* and *P. chrysogenum* (RsPc); *R. oligosporus* and *R. stolonifer* (RoRs); *A. niger and P. chrysogenum* (AnPc); *A. niger and R. stolonifer* (AnRs); *R. oligosporus and P. chrysogenum* (RoPc); unfermented mango kernel cake (UMKC).

Table 5. Effect of mixed-culture fermentation at ambient temperature (27±5ºC) on reducing sugars in "Oori" mango kernel cake.

Mixed-culture fermented MKC	Fermentation period (h)/Sugar content (mg/g)							
	0	24	48	72	96	120	144	168
RoAn	15.12[a]	14.71[ab]	16.17[ab]	16.43[ab]	18.36[cd]	20.96[c]	19.67[cd]	20.57[c]
RoAn	15.12[a]	15.25[abcd]	16.59[b]	17.30[bc]	17.67[bc]	18.47[b]	19.06[cd]	19.67[b]
RoRs	15.12[a]	14.65[a]	15.80[ab]	16.51[ab]	18.21[cd]	17.65[b]	16.80[b]	15.51[a]
AnPc	15.12[a]	15.86[d]	17.53[c]	18.41[c]	18.88[cd]	21.35[c]	21.13[e]	22.18[d]
AnRs	15.12[a]	15.43[bcd]	16.27[b]	16.93[b]	19.95[b]	19.10[b]	18.87[c]	19.34[b]
RoPc	15.12[a]	15.47[cd]	15.91[ab]	16.06[ab]	19.20[d]	18.24[b]	20.51[de]	20.78[c]
UMKC	15.12[a]	15.12[abc]	15.36[a]	15.49[a]	15.49[a]	15.37[a]	15.14[a]	15.17[a]
SEM	0.00	0.22	0.27	0.42	0.38	0.59	0.23	0.28

Values are means of three replicates determinations; SEM = standard error of mean; means within column having different superscripts differ significantly ($p < 0.05$); *R. oligosporus* and *A. niger* (RoAn), *R. stolonifer* and *P. chrysogenum* (RsPc); *R. oligosporus* and *R. stolonifer* (RoRs), *A. niger and P. chrysogenum* (AnPc); *A. niger and R. stolonifer* (AnRs), *R. oligosporus and P. chrysogenum* (RoPc); unfermented mango kernel cake (UMKC).

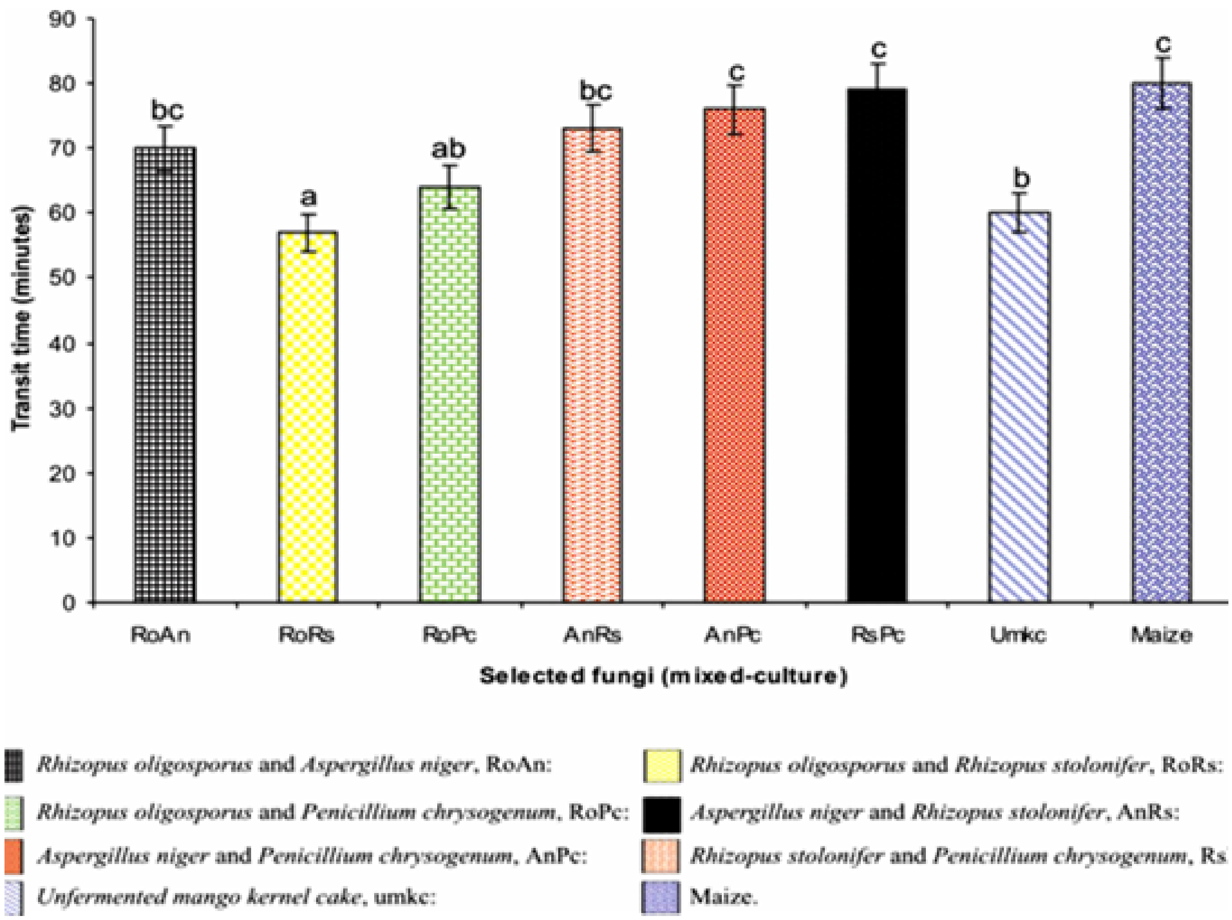

Figure 1. Transit time for the cockerels fed on mixed-culture fungal fermented “Oori” mango kernel cake.

Table 6. Nutrient retention of the cockerels fed on mixed-culture fungal fermented “Oori” mango kernel cake.

Mixed culture fermented MKC	Feed intake and apparent nutrient retention						
	Feed intake (g/bird/day)	Ether Extract (%)	Crude fibre (%)	Crude protein (%)	CHO (NFE) (%)	Energy retention (MJ/kg)[1]	ANR (%)
RoAn (T1)	28.34^{d}	70.39^{b}	19.44^{bc}	30.14^{b}	65.96^{bc}	11.88^{ab}	46.48^{b}
RoRs (T2)	21.90^{b}	81.59^{c}	23.34^{c}	55.01^{d}	77.96^{c}	15.02^{d}	59.45^{c}
RoPc (T3)	24.15^{bcd}	76.78^{bc}	39.41^{d}	49.09^{cd}	67.80^{bc}	14.49^{cd}	58.27^{c}
AnRs (T4)	23.43^{bc}	51.05^{a}	20.18^{bc}	35.61^{b}	63.69^{bc}	10.33^{a}	42.63^{ab}
AnPc (T5)	23.12^{bc}	77.23^{bc}	15.45^{b}	37.25^{b}	55.94^{b}	12.34^{b}	46.47^{b}
RsPc (T6)	27.74^{cd}	69.05^{b}	7.99^{a}	39.69^{bc}	37.52^{a}	10.63^{a}	38.56^{a}
UMKC (T7)	12.16^{a}	68.76^{b}	15.45^{b}	9.84^{a}	64.82^{bc}	10.34^{a}	39.72^{ab}
Maize (T8)	40.00^{e}	56.07^{a}	21.92^{c}	56.06^{d}	92.38^{d}	13.27^{bc}	56.61^{c}
SEM	1.14	2.02	1.40	2.71	3.55	0.34	1.63

NFE = Nitrogen free extract obtained by calculation; SEM = standard error of mean; ANR = average nutrient retention; means within column having different superscripts differ significantly ($p < 0.05$); *R. oligosporus* and *A. niger* (RoAn); *R. stolonifer* and *P. chrysogenum* (RsPc); *R. oligosporus* and *R. stolonifer* (RoRs); *A. niger and P. chrysogenum* (AnPc); *A. niger and R. stolonifer* (AnRs); *R. oligosporus and P. chrysogenum* (RoPc); unfermented mango kernel cake (UMKC).

those fed on *R. oligosporus* and *R. stolonifer* fermented MKC (T2) had lowest feed intake (21.90 g/day/bird).

The results of nutrient retention of the birds fed on the mixed-culture fermented MKC are shown in Table 6. The values of average nutrient retention (ANR) of the mixed-culture fermented MKC were significantly ($p < 0.05$)

higher than the values (39.72%) of unfermented MKC (T7); except for nutrient retention (38.56%) of T6 that was fermented with mixed-culture of *R. stolonifer* and *P. chrysogenum*. Highest ANR (59.45%) was obtained for the birds fed on *R. oligosporus* and *R. stolonifer* fermented MKC (T2). There was remarkable improvement in ANR (59.45%) of the MKC when *R. oligosporus* and *R. stolonifer* (T2) were combined as starter cultures compared with the values (35.39 and 50.39%) of their mono-culture (*R. oligosporus* and *R. stolonifer*), respectively (Kayode et al., 2009). However, ranking according to ANR of the birds fed on the test ingredients is as follows: RoRs (T2) > RoPc (T3) > Maize (T8) > RoAn (T1) > AnPc (T5) > AnRs (T4) > RsPc (T6).

Conclusion

In conclusion, the results showed that valuable nutrients like protein and glucose were increased in the mixed-culture fungal fermented MKC. There was improvement in transit time and significant ($p < 0.05$) increase in nutrient retention of the fungal mixed-culture fermented MKC compared with unfermented MKC. The results of the fermented MKC were comparable with maize used as control which makes it suitable as a potential feed ingredient in chicken's diet.

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