Effect of fermentation condition on proximate composition of rumen filtrate fermented wheat offal

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Abstract

The use of agro-industrial by products (AIBs) that has low nutritional significance to man has been suggested as one of the possible solutions for incessant high cost of feed ingredient in poultry feeding. Wheat offal is one of such AIBs with limited application in poultry feeding due to poor nutritional value. Fermentation is one of the method of improving the nutritional quality of feed stuff. The study was conducted to assess the effect of fermentation condition on proximate composition of wheat offal. The treatments were assigned to two inoculum concentration (50 vs 100%), three moisture levels (60, 80 and 100%) and four periods of fermentation (1, 2, 3 and 4 weeks) in a 2 x 4 x 6 factorial arrangement of completely randomized design. Data collected was subjected to analysis of variance, significant differences were separated using Duncan's multiple range test. The results of experiment revealed that wheat offal fermented for two weeks had the highest (P≤0.05) crude protein (21.53%) and ash (3.01%) while the lowest crude fibre (3.83%) and nitrogen free extract (63.51%) content were also recorded in this treatment. Wheat offal fermented at 100% moisture level recorded the lowest crude fiber (4.40%) and Nitrogen free extract (48.56%) values while the highest values for crude fiber and Nitrogen free extract were respectively were obtained for wheat fermented at 60% moisture level. The effect of interaction between fermentation duration, moisture level and inoculum concentration was significant (P≤0.01). It was concluded from the study that two weeks fermentation of wheat offal enhanced its nutritional value. Wheat offal can be fermented for 1- 2 weeks at 80 - 100% moisture level with 50 -100% inoculum concentration for optimum nutritional improvement

Keywords; wheat offal, nutritional value, fermentation duration, moisture level, inoculum concentration
Running title; Fermentation effect on wheat offal composition

Effet des conditions de fermentation sur la composition immédiate des abats de blé fermentés issus du filtrat ruminal

Résumé

L'utilisation de sous-produits agro-industriels (SAI), peu importants sur le plan nutritionnel pour l'homme, a été suggérée comme une solution au coût toujours élevé des ingrédients alimentaires pour l'alimentation des volailles. Les abats de blé font partie de ces SAI, dont l'application est limitée en raison de leur faible valeur nutritionnelle. La fermentation est une méthode permettant d'améliorer la qualité nutritionnelle des aliments. L'étude a été menée pour évaluer l'effet des conditions de fermentation sur la composition immédiate des abats de blé. Les traitements ont été répartis en deux concentrations d'inoculum (50 vs 100 %), trois taux d'humidité (60, 80 et 100 %) et quatre périodes de fermentation (1, 2, 3 et 4 semaines) selon un schéma factoriel 2 x 4 x 6 entièrement randomisé. Les données recueillies ont été soumises à une analyse de variance ; les différences significatives ont été distinguées par le test de Duncan. Les résultats de l'expérience ont révélé que les abats de blé fermentés pendant deux semaines présentaient les teneurs en protéines brutes (21,53 %) et en cendres (3,01 %) les plus élevées (p < 0,05), tandis que les teneurs en fibres brutes (3,83 %) et en extrait sans azote (63,51 %) étaient également les plus faibles. Les abats de blé fermentés à 100 % d'humidité présentaient les valeurs les plus faibles de fibres brutes (4,40 %) et d'extrait sans azote (48,56 %), tandis que les valeurs les plus élevées pour ces deux valeurs ont été obtenues pour le blé fermenté à 60 % d'humidité. L'interaction entre la durée de fermentation, le taux d'humidité et la

concentration d'inoculum était significative (p ≤ 0,01). L'étude a conclu qu'une fermentation de deux semaines des abats de blé améliorait leur valeur nutritionnelle. Les abats de blé peuvent être fermentés pendant une à deux semaines à un taux d'humidité de 80 à 100 % avec une concentration d'inoculum de 50 à 100 % pour une amélioration nutritionnelle optimale.

Mots clés : abats de blé, valeur nutritionnelle, durée de fermentation, taux d'humidité, concentration d'inoculum

Introduction

Monogastric animals cannot digest fibrous feed material and must rely on food grains consumed by man for their nourishment. The use Agro-industrial by-products (AIBs) that are readily available, inexpensive with low/ less direct nutritional relevance to humans have been suggested as one of the strategies to address the food/feed competition and the incessant high cost of poultry feeding. Wheat offal (WO) is one of the AIBs that is widely utilized as a conventional source of dietary fibre in poultry feed formulations as in Nigeria (Makinde, *et al*., 2017). However, its use in poultry diet (especially chickens) is limited as a result of low nutritional value and inherent anti nutritional factors called non-starch polysaccharides (NSPs). The NSPs have been implicated for depressing nutrient digestibility, absorption, availability and efficient utilization in monogastric animals (Alayande *et al.,* 2016). Nutritionists have recommended several processing methods like the use of exogenous enzymes and application of biotechnology methods of fermentation to enhance the nutritional value of AIBs (Aro *et al*., 2013; Ereke *et al*., 2017).

Fermentation is the chemical transformation of organic substances into simpler compounds by the activities of complex organic catalysts produced by microorganisms such as bacteria, and fungi (Madigan *et al.,* 2018). This process has been widely used to increase the bioavailability of nutrients and reduce the levels of anti-nutritional factors in food ingredients. (Naji *et al*., 2015, Elmasry *et al*., 2017). Fermentation process is affected by several factors such as moisture content, incubation time, incubation temperature, initial pH of the medium, inoculum size, type of inoculum, nature of substrate, type of micro-organism among others. Optimum combinations of major parameters are essential for successful solid state fermentation (Pandey, 2008. Akcan *et al*., 2012). According to Subramaniyam and Vimala, (2012) the outcome of fermentation varies for each substrate thus, it is important that fermentation procedures be optimized for each substrate as different organism react differently to each substrate. The objective of the study was to evaluate effect of fermentation conditions on the proximate composition rumen filtrate fermented wheat offal (RUFFWO)

Study Area

The study was conducted at the laboratory of the Department of Animal Science University of Maiduguri. Maiduguri is located between Latitude 11^0.85 and 12^0N and Longitude 13^0.16 and 14^0E, and at an altitude of 325m above sea level. The mean temperature ranges from 34 - 43^0C (April- May) and the lowest (23 - 28^0C) during the months of December to January. The area is characterized by short duration of rainfall (June to September) which varies from minimum of 300 mm to maximum of 700 mm per annum.

Sourcing, Processing and Preparation of Experimental Material

Wheat offal (WO) was purchased from local market in Maiduguri. Fresh cattle rumen content was collected immediately after slaughter at Maiduguri abattoir and was immediately filtered through a sieve. The residue was discarded and the filtrate was used to inoculate the WO at different concentrations and moisture levels (w/v).

Laboratory Studies

The laboratory study was conducted in two stages The first stage was to determine the optimum fermentation conditions (optimum moisture content, inoculum concentration and duration of fermentation while proximate analysis of the fermented wheat offal was conducted in the second stage.

Experimental design

The study was conducted in a completely randomized design in 2 x 3 x 4 factorial arrangement with three factors: Factor 1: Inoculum concentration (50 and 100%); Factor 2: Moisture level (60,80 and 100%) and Factor 3; Incubation time (7, 14, 21 and 28 days); All treatments were replicated three times.

Preparation of inoculants, moisture level and Substrate Inoculation

Two inoculum concentration levels (50 and 100%). were prepared as follows;

Inoculum A = 50% rumen filtrate + 50% water;

Inocuum B (100% rumen filtrate); the inoculants were used to inoculate the WO at different moisture levels. The quantity of inoculant to be used to wet a given quantity of wheat offal (WO) at different moisture levels are presented below.

Level of moisture required to wet 1000g WO

Sample 1	60%	=	1000g WO	+	600 mL	Inoculum A
Sample 2	80%	=	1000g WO	+	800 mL	Inoculum A
Sample 3	100%	=	1000g WO	+	1000mL	Inoculum A
Sample 4	60%	=	1000g WO	+	600mL	Inoculum B
Sample 5	80%	=	1000g WO	+	800mL	Inoculum B
Sample 6	100%	=	1000g WO	+	1000mL	Inoculum B

The wheat offal was thoroughly mixed with the inoculants at various proportions in a bucket before transferring them into fermentation tubes.

Fermentation

Empty bottles of equal volume were used to ferment the inoculated WO for varying number of days (7, 15, 21and 28 days). The bottles were properly labeled before initiation of the process. The inoculated wheat offal was properly compacted into the bottles, the mouth of which was sealed with polythene material and tightly closed with the covers to create anaerobic condition for fermentation at room temperature. Each of the sample was replicated in four and one set of the samples was opened each week which was sundried for two to three days and securely stored. At the end of each stipulated fermentation period, each bottle was opened and the content was sundried. Samples from each bottle was taken for proximate analysis according to AOAC (2010).

Proximate Analysis

Dry matter, crude protien, crude fibre, ether extract, nitrogen-free extract and ash content of the fermented wheat offal were determined according to AOAC (2010) methods of analysis.

Statistical analysis

Data collected was subjected to analysis of variance using the Statistical Analysis Software (SAS version 9.4). Significant differences was compared using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

The effect of fermentation duration, moisture level and inoculum concentration on proximate composition of RUFFWO is presented in Table 1. Results obtained from this study revealed significant ($P \leq 0.05$) effect of fermentation duration on proximate composition of RUFFWO. Wheat offal (WO) fermented for two weeks had significantly higher (21.53%) crude protien (CP) content followed by those fermented for one (18.95%) and four weeks (18.28%). The least CP (16.90) recorded at three weeks of fermentation was similar to that of one and three weeks. Ether extract (EE) values showed an increasing trend as fermentation time advances. Wheat offal fermented for one week had the lowest EE value

(6.87%) while those fermented for two, three and four weeks had higher values that were similar statistically (8.10-8.58%). Fermentation duration also had significant ($P \leq 0.05$) influence on crude fibre (CF). WO fermented for two weeks had significantly lower CF values (3.83%) than groups fermented for three, four and one week. Wheat offal fermented for one, three and four weeks were statistically similar and less than two weeks fermented wheat offal that had the lowest Nitrogen free extract (NFE) value (63.44%). The findings of this study is consistent with the reports of Iyayi and Aderolu (2004), Lateef *et al*. (2008) Lawal *et al*. (2012) and El-masry *et al*. (2017) that 2 weeks fermentation enhanced the crude protein and reduced crude fibre content of brewers dried grain (BDG), rice bran (RB), palm kernel meal (PKM), corn and wheat bran.

Studies by Ndams *et al*. (2008) revealed that brewers' dried grain fermented for 2-days had higher CP content than sample fermented for 8 days. The increased crude protein in the RUFFWO could be as a result of biodegradation of the fibre matrix in wheat offal to release the embedded nutrients. Decline of CP in week three could be as a result of proliferation of microorganism (Familade and Babayemi, 2009) that utilize substrate (especially protien and energy) for microbial biomass production (Oduguwa *et al.,* 2008; Bamigboye 2012).

Table 1. Effect of Fermentation Duration, Moisture Level and Inoculum Concentrations on Proximate Composition of rumen filtrate fermented wheat offal (RUFFWO)

	Nutrients (%)						
Parameters	Crude protein	Ether extract	Crude fibre	Ash	Moisture	NFE	Energy kcal/kg
Duration (weeks)							
1	18.95^{b}	6.87^{b}	5.30^{a}	2.65^{b}	16.08	66.23^{a}	2005.74
2	21.53^{a}	8.10^{a}	3.83^{b}	3.01^{a}	16.25	63.51^{b}	2110.69
3	16.90^{b}	8.35^{a}	5.06^{a}	2.53^{b}	15.74	67.14^{a}	2009.61
4	18.28^{b}	8.58^{a}	4.82^{a}	2.48^{b}	16.25	65.83^{a}	2160.08
SEM	0.51^{ns}	0.17^{ns}	0.15*	0.07^{ns}	0.10^{ns}	0.63*	11.66^{ns}
Moisture level (%)							
60	17.94	7.52	5.25^{a}	2.50	16.17	66.77^{a}	2067.06
80	19.24	8.14	4.61^{ab}	2.72	16.22	65.27^{ab}	2053.74
100	20.31	8.26	4.40^{b}	2.78	15.83	64.23^{b}	2093.74
SEM	0.51^{ns}	0.17^{ns}	0.15*	0.07^{ns}	0.10^{ns}	0.63*	11.66^{ns}
Inoculum concentration (%)							
50	19.02	8.08	4.91	2.74	16.09	65.23	1996.85
100	19.31	7.87	4.59	2.59	16.06	65.61	2146.19
SEM	0.53^{ns}	0.17^{ns}	0.15^{ns}	0.07^{ns}	0.11^{ns}	0.66^{ns}	11.13^{ns}

a,b,c Means on the same column having different superscripts are significantly different ($P \leq 0.05$)
SEM: Standard Error of Mean, NFE= nitrogen free extract, ns=not significantly different ($P > 0.05$)

The trend observed in this study is similar to the reports of Misra *et al*. (2007) and Ndams *et al.* (2008) who showed increase in CP, EE, and Ash and reduced CF of brewers dried grain and mustards straw (MS) with increase in incubation time which thereafter declined. Similarly, the reports of Ezekiel and Aworh (2013) reported increase in protein and total sugars with fermentation time. However, further increase in incubation period did not lead to increase in protein and total sugar content as observed in this study. This indicated that fermenting WO beyond two weeks may not have any advantage on its nutrient composition.

Effect of moisture level on proximate composition of RUFFWO

Results obtained from this study revealed significant ($P \leq 0.05$) effect of moisture level (ML) on the crude fibre and nitrogen free extract values of RUFFWO. The trend revealed decreasing levels of the nutrients as moisture level increased. The WO inoculated at 100% ML recorded the lowest CF (4.40%) and NFE (48.56%) **v**alues while the highest values for CF and NFE were obtained for WO inoculated at 60% ML followed by 80% ML. The finding of this study indicated that high moisture level is essential for bringing about optimum fibre reduction, though at the expense of NFE. This is expected because the fermenting bacteria required sufficient moisture and energy to multiply and for various physiological processes. The findings of this study is similar with the finding of Misra *et al*. (2007) who reported progressive increase in biodegradation of DM, NDF and ADF with increasing levels of moisture.

Effect of inoculum concentration on proximate composition of RUFFWO

There was no significant ($P>0.05$) effect of inoculum concentration on proximate composition of RUFFWO. This could suggest that diluted quantity of inoculant can cause similar fermentation effect as concentrated inoculant. This result is at variance with the reports of Ndams *et al*. (2008) who showed higher CP in brewers' dried grain of 10:2 substrate to inoculum concentration than sample on 10:4 concentration. Nuñez-Gaona *et al*. (2010) reported significant reductions in incubation time with increase in inoculum concentration while Jahwarhar *et al*. (2011) reported lower inoculum size (5.5%) as optimal for mannanase product compared to 10 and 15%.

Interaction effect of fermentation condition on proximate composition of RUFFWO

Two-way interaction

Two-way interaction effect of fermentation condition on proximate composition of RUFFWO is presented in Table 2

Duration x moisture level

There was a highly significant ($P \leq 0.01$) interaction effect between fermentation duration and moisture levels for the proximate composition of RUFFWO. In the first three week of fermentation, CP showed an increasing trend with increase in ML. The highest CP values were observed at 100% ML for the three weeks. Also EE values were higher at 80 and 100% ML from the second to the fourth week, the highest value was observed at 80% ML in the third week. Similarly, CF decreased with increase in ML from the first to the third week with the lowest value recorded at 80% ML in the second week. Ash was higher at 80 and 100% ML for the same period.

The highest value was observed at two weeks on 100% ML. NFE and energy were inconsistent. The highest NFE was observed at week 3 on 60% ML while the highest energy value was noted at 100% ML in the second week of fermentation.

Duration x inoculum concentration

For the first and third weeks, CP was higher at 100% IC while being higher at 50% IC in the second and fourth weeks. Energy values were higher at 100% IC in the first three weeks. The highest value was observed at 100% IC in the

second week. There was no interaction with other parameters.

Table 2. Two-way interaction of fermentation condition on proximate composition of rumen filtrate fermented wheat offal (RUFFWO)

Parameters		Nutrients (%)						Energy
		CP	EE	CF	Ash	Moisture	NFE	(kcal/kg)
Duration (weeks)	ML (%)							
1	60	18.49	7.56	5.65	2.55	16.41	65.74	2267.24
	80	19.13	6.55	5.00	2.67	16.63	66.64	1798.81
	100	19.24	6.52	5.25	2.68	15.16	66.30	1951.13
2	60	19.70	7.13	4.70	2.25	15.62	66.20	1851.96
	80	23.82	8.42	3.25	3.39	16.20	61.10	2149.17
	100	24.06	8.75	3.54	3.41	16.91	60.21	2330.98
3	60	13.59	6.60	6.40	2.44	16.27	70.95	2126.15
	80	18.44	9.38	4.48	2.56	15.65	65.11	1972.62
	100	18.66	9.08	4.29	2.61	15.30	65.34	1930.02
4	60	19.98	8.82	4.25	2.76	16.37	64.18	2022.97
	80	15.57	8.20	5.72	2.25	16.41	68.24	2294.42
	100	19.28	8.71	4.50	2.43	15.97	65.06	2162.81
SEM		0.38^{**}	0.09^{**}	0.10^{**}	0.06^{**}	0.08^{**}	0.37^{**}	10.38^{**}
Duration (weeks)	I/C (%)							
1	50	17.23	7.15	5.66	2.67	16.01	67.27	1998.63
	100	20.67	6.60	4.93	2.59	16.12	65.19	2012.81
2	50	23.02	8.14	4.03	3.21	16.30	61.59	1955.67
	100	22.04	8.06	3.64	2.82	16.19	63.42	2265.74
3	50	15.73	8.03	5.54	2.22	15.61	68.40	1806.41
	100	18.07	8.61	4.58	2.85	15.87	65.87	2212.77
4	50	20.09	8.95	4.41	2.85	16.43	63.68	2226.66
	100	16.47	8.20	5.23	2.10	16.07	67.97	2093.46
SEM		0.40^{**}	0.14^{ns}	0.13^{ns}	0.06^{ns}	0.11^{ns}	0.53^{ns}	10.50^{**}
I C (%)	ML (%)							
50	60	17.44	7.28	5.12	2.75	15.90	67.38	2156.24
	80	19.30	8.36	4.65	2.81	16.12	64.86	1864.01
	100	20.31	8.60	4.96	2.65	16.23	63.46	1970.28
100	60	18.44	7.77	5.37	2.24	16.43	66.16	1977.92
	80	19.19	7.91	4.58	2.62	16.32	65.69	2243.50
	100	20.31	7.93	3.83	2.91	15.43	64.99	2217.17
SEM		0.53^{ns}	0.17^{ns}	0.14^{ns}	0.07^{ns}	0.10^{ns}	0.65^{ns}	10.41^{*}

a,b,c Means on the same column having different superscripts are significantly different ($P \leq 0.01$), ns= not significant ($P>0.01$), SEM: Standard Error of Mean, NFE= nitrogen free extract, IC=inoculum concentration; ML=moisture level CP= crude protien; CF= crude fibre; EE=ether extract ; NFE= nitrogen free extract;

Numerically however, EE values were lower at 100% ML in the first, second and forth weeks. The highest value was observed at 50% IC in the fourth week. CF values were lower at 100% IC

from the first to the third week. The lowest value was recorded at 100% IC in the second week.

The findings of this study are consistent with the reports of Ndams *et al.* (2008) who revealed no significant effect of IC on fermentation duration.

Inoculum concentration x moisture level

There was no interaction between inoculum concentration and moisture level.

Three-way interaction

Three -way interaction effect of fermentation condition on proximate composition of RUFFWO is presented in Table 3.

There was very highly significant ($P \leq 0.001$) interaction effect between fermentation duration, moisture level (ML) and inoculum concentration (IC) on proximate composition of RUFFWO.

In the first and third weeks of fermentation, CP was higher in the 100% IC at 60 and 100% MLs while being higher at 50% IC at all MLs in the second and fourth weeks. Suggesting that high IC could be used to achieve CP improvement within short fermentation duration of one week at minimal ML While EE was higher at 50% IC at 80 and 100% MLs for all weeks, CF was lower at 100% IC. Ash values were higher at 50% IC in the first, second and fourth weeks of fermentation at all MLs. NFE was higher at 100% IC for all MLs in the second and fourth weeks while it was higher at 50% IC at the 60 and 80% MLs for the first and third weeks. The highest CP and ash values were recorded in WO fermented with 50% IC at 80% ML for two weeks. While the highest EE value was observed at three weeks with 80% ML and 50% IC, CF was lowest at 80% ML on 100% IC in the second week. This observation could suggest that nutrient improvement may be achieved at higher IC, lower ML for short duration or at lower IC and ML for longer duration. Therefore, the findings of this study signifies the possibility of including higher levels of WO in the diet of monogastric animals especially broiler chickens. This can contribute to decrease the quantity of grains used in feed formulation, reduce food /feed competition and cost of poultry feeding. Thus, enhancing the sustainability of the poultry enterprise and supply protein to the expanding population.

Conclusion

It was concluded from this study that fermentation of wheat offal improved its crude protien, ether extract and ash content and caused a reduction in crude fibre content. Fermentation of wheat offal for 1- 2 weeks at 80 - 100% moisture level with 50 -100% inoculum concentration was reecommended for optimum nutritional improvement.

Table 3. Three way interaction of fermentation duration, moisture level (ml) and inocolum concentration (ic) on proximate composition of RUFFWO

Parameters			Nutrients (%)						
Duration (weeks)	ML (%)	IC (%)	CP	EE	CF	Ash	Moisture	NFE	ME (kcal/kg)
1	60	50	14.13	8.45	6.00	2.77	16.00	68.64	2527.49
		100	22.85	6.67	5.30	2.32	16.82	62.85	2006.98
	80	50	16.35	6.35	5.00	3.00	16.05	69.30	1461.45
		100	21.92	6.75	5.00	2.35	17.21	63.98	2136.17
	100	50	21.23	6.65	6.00	2.25	16.00	63.87	2006.98
		100	17.25	6.40	4.50	3.11	14.33	68.73	1895.27
2	60	50	20.36	6.50	4.96	2.50	15.34	65.68	1674.31
		100	19.05	7.76	4.45	2.00	15.90	66.73	2029.61
	80	50	24.50	8.96	3.51	3.58	16.55	59.45	2095.55
		100	23.15	7.88	3.00	3.20	15.86	62.76	2202.78
	100	50	24.20	8.96	3.62	3.57	17.01	59.64	2097.15
		100	23.93	8.55	3.47	3.26	16.81	60.78	2564.80

3	60	50	11.31	5.55	6.55	2.35	15.33	74.24	2098.36
		100	15.88	7.65	6.26	2.53	17.21	67.67	2153.93
	80	50	19.55	9.51	4.65	2.33	15.81	63.95	1560.01
		100	17.34	9.25	4.32	2.80	15.50	66.28	2385.23
	100	50	16.33	9.21	5.44	2.00	15.70	67.01	1760.90
		100	21.00	8.95	3.15	3.22	14.90	63.67	2099.18
4	60	50	23.98	8.64	3.00	3.40	16.95	60.98	2324.79
		100	15.98	9.00	5.50	2.12	15.80	67.39	1721.14
	80	50	16.80	8.65	5.45	2.35	16.10	66.75	2339.01
		100	14.35	7.76	6.00	2.15	16.72	69.73	2249.83
	100	50	19.49	9.57	4.80	2.81	16.24	63.32	2016.18
		100	19.08	7.85	4.21	2.05	15.70	66.80	2309.48
SEM			0.02^{***}	0.00^{***}	0.02^{***}	0.00^{***}	0.00^{***}	0.01^{***}	0.01^{***}	0.09^{***}

a,b,c *** =very highly significant (P≤0.01), SEM: Standard Error of Mean, CP= crude protien; CF= crude fibre; EE=ether extract ; NFE= nitrogen free extract; RUFFWO = Rumen Filtrate Fermented Wheat Offal

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