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Effects of sprouted sorghum *(Sorghum bicolor)* diets fortified with exogenous enzymes on egg production in Red and White Amberlink layers

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Abstract

The aim was to evaluate the effects on egg production of feeding raw versus sprouted sorghum (*Sorghum bicolor)*–soybean *(Glycine max)* oil cake diets fortified with exogenous enzymes to red and white Amberlink layers (16 w in production). Birds were stocked at three birds per cage in an open house. The 6-w experiment was conducted in a randomized 3 (diets) × 2 (enzymes) × 2 (strains) factorial design replicated six times. A commercial late lay product was the positive control for iso-nutrient, sprouted, and raw sorghum-based soybean test diets. Duplicates of all diets were fortified with 500 g/tonne of a multi-enzyme cocktail. Raw sorghum resulted in low feed intake. High laying rates were attained in red layers on the enzyme-fortified commercial diet, white Amberlink layers on the same diet without enzymes, and in both strains on the enzyme-fortified, sprouted sorghum diet. Raw sorghum without enzymes resulted in low laying rates across strains. The laying rate and feed conversion decreased: commercial feed >sprouted sorghum >raw sorghum. Enzymes reduced egg weight in red layers on the sprouted sorghum diet, with an opposite effect in white layers on the commercial diet. Compared to the standard diet, the sorghum diets reduced feed intake and egg production and increased the feed conversion ratio, with better relative egg output (95%) on the sprouted, compared to the raw (85%), sorghum diet. Treatment interactions on the laying rate (strain × diet × enzyme), and egg size (strain × enzyme) suggest that the potency of exogenous enzymes depends on the layer strain and diet.

Keywords: Exogenous enzymes, feed alternatives; sprouting, broiler nutrition

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Introduction

The cost of conventional poultry feeds continues to escalate, typically contributing up to 75% of the total cost of production (Alagawany *et al*., 2018). To maintain enterprise viability, high feed costs necessitate high precision feeding of least-cost formulated diets. The nutrient balance of the conventional maize–soybean poultry diet hinges on the nutrient content and complementarity of these basal ingredients. Substituting them with alternatives risks nutritionally-inferior diets, a scenario which requires careful ingredient selection and effective processing to maintain efficacy of the conventional diets.

Maize is quantitatively and economically a major component in the poultry diet. Its declining, erratic global production is driving cost (Mabelebele *et al*., 2015). In sub-Saharan Africa over the past few decades, genetic improvement for high yields and superior quality and extension programmes have been designed to expand maize production, often at the expense of the more climate-resilient traditional cereals (Mwadalu & Mwang, 2013). Given the limited irrigation capacity in these regions, viable dry-land maize production is largely limited to areas which receive reliable, high rainfall (Travis *et al*., 2006). However, climate-change is disrupting both the spatial and temporal distribution of rainfall and diminishing the ecosystems which were previously

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considered suitable for maize production (Ringler *et al.*, 2011; Ayanlade *et al.*, 2018). Concurrently, demand from an increasing human population is a strong push on the cost of maize (Linden, 2017).

In tropical, developing regions, the consensus strategy to mitigate declining maize production is to promote the climate-resilient small grains (Muzerengi & Tirivangasi, 2019). A suitable candidate is sorghum (*Sorghum bicolor*) (Sedghi *et al*., 2011). In small-scale poultry production, compared to the millets, sorghum offers advantages such as relative nutritive value (Nyamambi *et al*., 2007), adaptability to diverse soil and climatic conditions (Sedghi *et al*., 2011), and higher yield in relation to the production cost (Dicko *et al*., 2006). For poultry feeding, compared to maize, the relative nutritional value of both current and new sorghum varieties requires continuous evaluation. Previously, Sedghi *et al*. (2011) reported a comparable nutritive value to maize (13.7 MJ ME kg^{-1}, 9.5% CP versus 13.9 MJ ME kg^{-1}, 10.1% CP, respectively). Despite an acceptable nutrient profile and the presence of bioactive phenolic acids and flavonoids, sorghum contains high levels of soluble non-starch polysaccharides and condensed tannins (Dicko *et al*., 2006). Whole sorghum protein is also dominated (70%) by kafirins, which are relatively poorly-digested proteins due to a high degree of polymerization, extensive disulphide bridges, and strong interaction with condensed tannins and resistant starches (Belton *et al*., 2006) and phytate (Liu *et al.*, 2015). These antinutritive attributes may affect dietary efficacy in terms of both energy and protein utilisation (Liu *et al.*, 2015).

Given the antinutrients, effective sorghum processing is critical to mitigate its nutritional limitations. In small holder systems, germination is considered a practical, cost-effective, biologically-efficient bioprocess (Correia *et al*., 2008; Inyang & Zakari, 2008). Subject to the dietary chemical matrix and depending on the age and strain of the birds (Cowieson *et al*., 2006), exogenous enzymes might further enhance the dietary efficacy and stimulate greater production and commercialisation into a viable value chain to support more economically- and environmentally-sustainable poultry production. The efficacy of novel diets and their processing is potentially subject to unique, as yet undefined, layer strain nutritional traits. Therefore, this study investigated the effects of sprouting sorghum and fortification of a sorghum-based diet with a cocktail of xylanase, glucanase, and phytase enzymes on red and white Amberlink layer performance.

Material and Methods

Ethical approval for the study was granted by the University of Venda Ethics Committee (SARDF/19/ANS/04/1305). All housing, feeding, and management practices adhered to statutory standards for rearing laying birds in battery systems. The study was conducted at the University of Venda, School of Agriculture Experimental Farm, situated at latitude 22°58′32″ South, longitude 30°26′45″ East, at an altitude of 596 m.

Red sorghum (*Sorghum bicolor*) grain was cleaned and screened for viable seeds. The grain was divided into two equal portions. One portion was soaked in a 2% sodium hypochlorite solution for 30 min for sterilisation, followed by a 12-h soak in tap water. The grain was then spread on perforated garden plastic sheeting placed on elevated steel screens for 5 d with intermittent irrigation. Sprouting was terminated by sun-drying the sprouts while on spread black plastic laid on a concrete slab. Representative samples were collected for chemical analyses.

Enzyme-treated diets contained 500 g/tonne of a custom multi-enzyme cocktail (Chemunique (Pty) Ltd, Lanseria South Africa, Product CHE/XBP 600) which contained endo-1,4-beta-xylanase (EC-3.2.1.8-2440 U/kg)**,** endo-1,3(4)-beta-glucanase (EC-3.2.1.6-304 U/kg), and 6-phytase (IUB 3.1.3.26-1220 U/kg). Three experimental diets were used: a commercial control diet (Meadow Feeds (Pty) Ltd, Powerlay Late Lay; product V16418), a raw sorghum–soybean diet, and a sprouted sorghum–soybean diet. The ingredient and chemical composition of the test diets is presented in Tables 1 and 2. The diets were formulated to be iso-nutrient and met the minimum feeding standards for layer diets (NRC, 1994). Chemical analyses were conducted according to AOAC (1990) standards. Dry matter content was determined using method 930.15, ash content by method 942.05, nitrogen content via the Kjeldahl procedure (method 984.13), and fat content by Soxhlet extraction (method 930.15).

Table 1 Ingredient composition (% as is) of sorghum diets

Ingredients	[3]Diets	
	Raw sorghum	[1]Sprouted sorghum
Soybean cake	12.13	12.34
Raw sorghum	72.35	-
[1]Sprouted sorghum	-	72.40
[2]Layer macro pack	2.88	2.90
Lysine	0.09	0.09
Limestone	10.37	10.84
Mono Dicalcium Phosphate	1.73	0.97
Sand	0.46	0.46
Total	100.00	100.00

[1]30-min sterilisation in 2% aqueous sodium hypochlorite, 12 h soaking in tap water, 5-d open-air sprouting, sun-dried
[2]Composition per kg of premix: Vitamin A, 588235.300 IU; Vitamin D3, 205882.400 IU; Vitamin E, 882.353 IU; Vitamin K, 88.235 mg; Vitamin B, 117.647 mg; Vitamin B2, 235.294 mg; Vitamin B6, 147.059 mg; Niacin, 1647.059 mg; Calcium Pantothenate, 411.765 mg; Biotin, 1470.588 µg; Folic acid, 29.412 mg; Vitamin B12, 1176.471 µg; Choline Chloride, 15278.820 mg; 6-phytase, 17647.060 FTU; Limestone (as carrier), 32.353 g; Salt, 235.294 g; Dicalcium phosphate, 470.588 g; Cobalt from cobalt sulphate, 29.412 mg; Copper from Copper sulphate, 352.941 mg; Iron from Ferrous sulphate, 1764.706 mg; Iodine (potassium iodide), 58.824 mg; Manganese (manganese sulphate), 4117.647 mg; Zinc (zinc sulphate), 1764.706 mg; Selenium (sodium selenite), 8.824 mg; lysine, 88.824 g; Methionine, 109.412 g; Enzyme (Natuphos E 10000 G), 1.765 g
[3]Enzyme-treated diets contained 500 g/tonne of a custom multi-enzyme cocktail (Chemunique (Pty) Ltd, Lanseria South Africa, Product CHE/XBP 600) which contained endo-1,4-beta-xylanase (EC-3.2.1.8-2440 U/kg)**,** endo-1,3(4)-beta-glucanase (EC-3.2.1.6-304 U/kg), and 6-phytase (IUB 3.1.3.26-1220 U/kg)

Table 2 Analysed ingredient and calculated dietary chemical composition

	DM	Fat	ADF	NDF	CP	Ca	P
Ingredients (g kg $^{-1}$ DM)							
Raw Sorghum	923.1	31.5	97.0	307.9	98.4	0.2	0.3
[1]Sprouted Sorghum	916.7	28.3	118.5	244.9	142.6	0.3	0.41
Soybean Cake	940.3	22.2	153.7	297.4	485.2	2.5	0.64
[2]Diets (g kg $^{-1}$ DM)							
Raw Sorghum	934.0	25.4	258.0	88.6	131.2	45.1	5.1
[1]Sprouted Sorghum	931.1	23.0	211.6	103.6	130.4	45.1	4.9

[1]30-min sterilisation in 2% aqueous sodium hypochlorite, 12 h soaking in tap water, 5-d open-air sprouting, sun-dried
[2]Enzyme-treated diets contained 500 g/tonne of a custom multi-enzyme cocktail (Chemunique (Pty) Ltd, Lanseria South Africa, Product CHE/XBP 600) which contained endo-1,4-beta-xylanase (EC-3.2.1.8-2440 U/kg)**,** endo-1,3(4)-beta-glucanase (EC-3.2.1.6-304 U/kg), and 6-phytase (IUB 3.1.3.26-1220 U/kg)

Egg production was evaluated in a trial running for 42 d using 216 red and white Amberlink layers that were 16 w into production. The layers were housed in a naturally-ventilated battery house in forty-eight cages (45 cm length × 45 cm width × 42 cm height), stocked at three birds/cage in a balanced, completely randomised 3 (diets) × 2 (enzymes) × 2 (strain) factorial with six replicates per treatment. Each pen was equipped with one nipple drinker and a width-long tube feeder. The trial was conducted under *ad libitum* feeding and automated 16:8 light:dark hour lighting regime. The birds received the recommended vaccination regimes for Newcastle, Infectious Bronchitis, and Infectious Bursal Diseases. Weekly cage feed intake, egg numbers, and weights were recorded, and the feed conversion ratio calculated per dozen and per kilogram eggs. Fresh eggs were collected, weighed and counted per cage daily at 09:00.

Data were checked for normality and homogeneity of variances and analysed using the General Linear Model (GLM) procedures in Minitab 18 (2017) for a randomized 3 (diet) × 2 (enzyme) × 2 (strain) factorial design, blocked by week of production. *Post hoc* comparisons of different treatment means were performed using Tukey's test at a significance level of $P < 0.05$.

Results

The results are presented in Table 3. Layers on the raw sorghum diet had low intake (P <0.05). The red Amberlinks laid heavier (P <0.05) eggs, with strain × enzyme interaction (P <0.026), due to a quantitative, greater enzyme efficacy in the red Amberlink. Strain × diet × enzyme was evident for laying rate (P = 0.0038). The highest (P <0.05) laying rate was obtained when the red Amberlink layers were on the commercial diet with enzymes and when white Amberlink layers were on the same diet without enzymes, similar to when both strains were on the enzyme-supplemented, sprouted sorghum diet (P >0.05). Low (P <0.05) laying rates were observed when both strains were on the raw sorghum, without any effect of enzyme (P >0.05). Laying rate was intermediate *(P <0.05)* in layers on the sprouted sorghum diet, without effect of the enzyme (P >0.05). The net effect was laying rate in the order: commercial feed >sprouted sorghum >raw sorghum *(P <0.05)*. Expressed on an egg number and egg weight basis, the FCR was in the order: commercial feed >sprouted sorghum >raw sorghum (P <0.01). Strain × enzyme interaction occurred for egg weight *(P <0.05)*, whereby the enzymes reduced *(P <0.05)* egg weight in red Amberlink layers when on the sprouted sorghum diet, which was similar on all other treatments, except for an opposite, quantitative enzyme effect on white Amberlink layers on the commercial diet.

Discussion

Apart from feed, the environment (Xu *et al*., 2022) and genetics (Liu *et al*., 2019) are key influences on the productivity of laying hens. This study hypothesised that sprouting of sorghum and dietary fortification with exogenous enzymes may differentially impact egg production in red and white Amberlink layer strains, which are both produced in local farming systems. Dekalb Poultry specifies that the red Amberlink strain has a larger body weight (1950 g vs. 1725 g) and higher feed intake (112 g/day vs. 108 g/day) compared to the white Amberlink strain, resulting in a slightly higher feed conversion ratio (FCR) (2.12 vs. 2.03). Moreover, the red Amberlink strain exhibits larger egg size (60.0 g vs. 62.1 g) and slightly higher hen-housed egg production (479 eggs vs. 486 eggs) per cycle. The experimental setup was typical of environmentally-uncontrolled, small-scale production, in which egg production may not be optimum to match the genetic potential of either strain. However, overall, egg production parameters approximated these strain production standards.

Feed intake is a crucial factor influencing egg production (Li *et al*., 2011). The impact of dietary inclusion on egg production largely depends on the tannin content of the sorghum, its processing, and the level of inclusion in relation to the poultry genotype (Singh *et al*., 2003). In the current study, the inclusion of raw sorghum in the diet led to reduced feed intake, likely due to the presence of tannins. Similar findings have been reported in previous studies (Manu-Barfo *et al*., 2013; Nortey *et al*., 2013). Agunbiade *et al*. (2016) reported low intake of sorghum-based diets despite enzyme fortification, which among other limitations, suggests the presence of harmful levels of residual tannins despite processing.

In the current study, egg production was in the order: commercial feed >sprouted sorghum >raw sorghum, which confirmed inferior sorghum diet efficacy and demonstrated the benefit of sprouting sorghum. Sprouting is widely reported to increase the nutritive value of cereal grains (Muhammad *et al*., 2013). In sorghum, the benefit is largely attributed to its reduction of tannins (which increases intake) and the digestibility of protein, with enhanced bioavailability of amino acids (Baba *et al*., 2012). In brown layers, Ochieng *et al*., (2018) observed similar egg weights when maize was replaced by graded quantities of low tannin sorghum. Similarly, in white Leghorn hens, Abera *et al*. (2020) reported improved egg production and feed efficiency when raw, improved sorghum varieties replaced maize. In the current study, significant interactions on egg production of the layer strain with the dietary factors (diet type, enzymes) were observed.

The laying rate and the egg output (numerically) were high when both the red and white Amberlink layers were on the commercial diet, regardless of supplementary enzymes, which suggests no benefit of enzymes on the dietary matrix. Egg production on the enzyme-supplemented, sprouted sorghum diet matched the commercial diet, which implies an additive benefit of sprouting sorghum and supplementary enzymes. Egg production was low on the raw sorghum, regardless of enzyme fortification, confirming the inferior quality of the raw sorghum diet and no response to the supplementary enzymes, likely the effect of antinutrients. In the current study, feeding layers raw or sprouted sorghum diets did not affect egg size, much as sprouting of sorghum and enzymes did not benefit the egg size. Expressed on both a quantitative (egg number) and qualitative (egg weight) basis, the FCR was in a similar order: commercial feed < sprouted sorghum < raw sorghum, which also confirmed the relative low efficacy of the sorghum diet and the improvement when sprouting the sorghum. A strain × diet × enzyme interaction occurred in a similar pattern for the FCR when expressed both on an egg weight and egg number basis. The highest FCR was observed in red and white Amberlink layers on the raw sorghum diet, regardless of the enzyme supplement. The lowest FCR was observed for the red and white Amberlink layers on the commercial diet, followed by both strains on sprouted-sorghum diets, with intermediate values for the rest of the treatments. The strain and dietary effects were regardless of the enzyme supplement.

This pattern of effects suggests less dietary efficacy in terms of FCR for the sorghum diets, with benefit from sprouting the sorghum but no overall benefit from enzymes on the efficiency of feed utilisation.

Table 3 Effects of feeding sprouted sorghum diets fortified with exogenous enzymes on egg production of red and white Amberlink layers

Treatments	Feed Intake	Egg Production				
		Rate	Mean weight	Output	Feed Conversion Ratio	
	(g/bird/day)	(%)	(g)	(g /bird/day)	(kg/dozen)	(kg/kg)
Strain						
Red	113.86	82.94	61.15[a]	50.68	1.69	2.30
White	113.70	82.08	60.27[b]	49.35	1.71	2.36
SEM	4.914	12.232	0.246	7.141	0.308	0.413
Diet						
Commercial	114.46[a]	88.05[a]	60.45	53.13[a]	1.59[c]	2.18[c]
Sprouted Sorghum	114.18[a]	83.63[b]	60.64	50.66[b]	1.68[b]	2.30[b]
Raw Sorghum	112.70[b]	75.84[c]	61.04	46.25[c]	1.83[a]	2.51[a]
SEM	0.486	1.116	2.490	0.659	0.0291	0.0393
Enzyme						
+	113.81	83.33	60.45	50.32	1.68	2.31
-	113.75	81.68	60.97	49.71	1.72	2.35
SEM	4.915	12.211	2.484	7.165	0.307	0.414
P Values						
Strain	0.776	0.551	0.002	0.115	0.551	0.224
Diet	0.027	0.001	0.244	0.001	0.001	0.001
Enzyme	0.910	0.252	0.074	0.472	0.264	0.488
Strain x Diet	0.838	0.314	0.775	0.345	0.442	0.495
Strain x Enzyme	0.728	0.861	0.026	0.601	0.965	0.564
Diet x Enzyme	0.944	0.310	0.507	0.479	0.504	0.659
Strain x Diet x Enzyme	0.925	0.038	0.484	0.098	0.068	0.106

[abcdef]Means in the same column that are not sharing a common superscript are significantly different (P <0.05)
SEM- standard error of the mean
[1]Meadow Feeds (Pty) Ltd, Delmas, South Africa Powerlay Late Lay diet, product V16418
[2]Enzyme-treated diets contained 500 g/tonne of a custom multi-enzyme cocktail (Chemunique (Pty) Ltd, Lanseria South Africa, Product CHE/XBP 600) which contained endo-1,4-beta-xylanase (EC-3.2.1.8-2440 U/kg)**,** endo-1,3(4)-beta-glucanase (EC-3.2.1.6-304 U/kg), and 6-phytase (IUB 3.1.3.26-1220 U/kg)
[3]30-min sterilisation in 2% aqueous sodium hypochlorite, 12 h soaking in tap water, 5-d open-air sprouting, sun-dried

While previous studies (Pasquali *et al*., 2017; Gidago *et al.*, 2020) suggest multi-enzyme efficacy in raw sorghum-based broiler diets, enzyme supplements to either raw or germinated sorghum did not benefit broiler chick performance (Torki *et al*., 2007). In the current study, the strain × enzyme interaction was significant for egg weight. The enzymes reduced egg weight in red Amberlink layers when on the sprouted sorghum diet, which was numerically similar on all other treatments, except for an opposite, numerical enzyme effect on white Amberlink layers on the commercial diet. Interactions on the laying rate and egg size resulted both beneficial and deleterious enzyme action, likely the effects of unique dietary chemical matrices. Across studies, the findings on enzyme benefit are inconsistent. Two previous studies (Pasquali *et al*., 2017; Gidado *et al*, 2020) suggested multi-enzyme efficacy in raw sorghum-based broiler diets. In contrast, enzyme supplements to either raw or germinated sorghum did not benefit broiler chick performance (Torki *et al*., 2007). These contradictory findings confirm the need for correct matching of the chemical matrix, optimized enzyme dosages, and considerations for the type, age, and strain of the birds (Cowieson *et al*., 2006). In principle, however, exogenous enzymes should increase the efficiency of sorghum digestion through breaking down of the anti-nutritional factors, such as fibre, phytate, and non-starch polysaccharide (NSP) (Adeola & Cowieson, 2011) to ensure

consistent animal performance regardless of dietary chemical variations (Flores-Cervantes *et al*., 2011; Lu *et al*., 2013).

Conclusions

The study assessed the effects of using raw versus sprouted sorghum as a maize substitutes on egg production in red and white Amberlink layers and the role of exogenous enzymes. Feeding raw sorghum decreased feed intake and egg production, likely due to its high tannin content, which impaired both feed intake and efficiency. Sprouting the sorghum improved feed intake and egg production, likely due to reduced tannin levels and enhanced nutrient availability. Enzyme supplementation had a synergistic effect on the sprouted sorghum diet in terms of egg production and feed conversion. In contrast, enzymes had no benefit on raw sorghum, probably because of high levels of anti-nutritional factors. The effect of enzymes on egg weight depended on the strain, with reduced egg weight in red Amberlink layers and an opposite effect on white Amberlink layers, indicating strain-specific enzyme responses and potential for tailored enzyme applications. Although layers fed the sprouted sorghum diets performed better than on the raw sorghum, the efficacy was less than the maize-based diets. Further research is recommended to assess the cost-effectiveness of sprouted sorghum with dietary improvement using enzyme fortification and the formulation of strain-specific enzyme cocktails.

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Author Contributions

The authors were jointly responsible for the conception, methods, including the design and execution of the experiment. MNR analysed the results; all authors jointly interpreted and drafted this manuscript.

Declared Conflict of Interest

The authors declare no conflict of interest in this study.

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