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Incorporation of dried goat rumen contents in layer diets improves egg yolk colour and acceptability of eggs

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Abstract

The use of dried goat rumen content (DGRC) as a partial replacement for fish meal in layer diets was investigated. A total of 90 H&N Brown Nick layer chickens were offered diets in which DGRC were incorporated at 0, 5 and 10% levels. Iso-caloric and nitrogenous diets were formulated to meet the recommended nutritional requirements for laying hens. Experimental birds were assigned to 9 cages (10 birds/cage) and experimental diets offered in a completely randomized design (CRD) with three replications. Data was collected on egg production, sensory characteristics of the eggs, and a partial budget analysis was undertaken. Diet significantly (P < 0.05) affected average daily feed intake (ADFI) and feed conversion ratio (FCR). There was an increase in ADFI and FCR with increasing levels of DGRC in the diets, laying percentage did not differ in layers fed on 0 and 5% DGRC diets (P > 0.05). Eggs from layers offered 10% DGRC were more acceptable than those of layers fed on 0 and 5% diets. A significant effect (P < 0.05) of treatments on yolk colour was observed. Eggs from 10% DGRC diets had more deep yellow yolks than eggs from 0 and 5% diets. It was concluded that use of DGRC in layer diets improved yolk colour, acceptability of the eggs and marginal rate of return (MRR).

Keywords: consumer preference, digestibility, egg production, feed conversion ratio, growth

1 Introduction

Inclusion of eggs in people's diets is considered a good way to access animal protein among households (Exler *et al.*, 2013) and also a key pillar in fighting hunger and malnutrition among communities (WHO, 2007). In Uganda, almost all households (HH) practice backyard chicken rearing in order to have access to animal protein or as a source of income to solve immediate domestic problems (Mwesigwa *et al.*, 2020). Commercial egg operations which are key to supplying the population with affordable eggs for nutritional enhancement are constrained by sky rocketing feed costs (Deepika *et al.*, 2018). This not only jeopardizes their efficient operation but also the ability to meet consumers' egg demands. Feed supply to the poultry industry, especially of quality protein sources, is still a daunting challenge in most developing countries. This is partly due to competition for these high quality protein sources such as fish meal with humans (Anderson et al., 2017). This increases demand for silver fish, an important animal protein source used in livestock feeds (Mwesigwa et al., 2013; Anderson et al., 2017). In Uganda, silver fish is mainly sourced from Lake Victoria and Lake Albert, however, due to its high demand, silver fish meal has been subjected to rampant adulteration, mostly with lake sand (Shahid & Talat, 2005). Use of adulterated silver fish meal in poultry diets not only leads to poor performance of the birds but also to a loss of revenue to both the farmer and the government. Therefore, the complete or partial replacement of fish meal with alternative feed ingredients would be a great relief to the poultry industry (Alagawany et al., 2019). Substantial levels of minerals and relative good crude protein content are found in rumen contents, which are almost freely available in most abat-

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toirs (Agbabiaka *et al.*, 2012; Elfaki *et al.*, 2015). This might therefore be a feed resource that should be evaluated as an alternative to fish meal in poultry diets. Its use will not only reduce the over-reliance on an animal protein source (silver fish) in Uganda, but might also mitigate environmental pollution (Bambatunde *et al.*, 2017; Uddin *et al.*, 2018). However, there is little information available guiding its utilisation as a resource in poultry feeds. Therefore, this study investigated the effect of using dried goat rumen contents (DGRC) as a partial substitute of fish meal in layer diets on egg production, egg quality and consumer egg acceptability as well as economic viability.

2 Materials and methods

2.1 Study site

The study was conducted at Mukono Zonal Agricultural Research and Development Institute (MUZARDI). It is located in Ntawo parish, Mukono Municipality, in Mukono district, at about 27 kilometres from Kampala on the northern shores of Lake Victoria (00°20'N, 32°45'E). The area lies 1161 m above sea level and has a tropical type of climate. The area receives 1390 mm of rainfall annually, and the mean annual temperature is 21.5 °C (70.7 °F).

2.2 Layer chicken management

Three months old layer chickens (H&N Brown Nick) were purchased from Biyinzika poultry International Ltd., an established poultry breeding company in Uganda. Prior to purchase of experimental birds, pens, watering system, feeding troughs and laying nests of the experimental house were properly cleaned and disinfected using Virkon® S disinfectant. The floor of each pen was covered with saw-dust as litter material to a depth of 3 cm. A total of 90 hens were used for the study and were identified using numbered wing bands. The experimental hens were weighed using a top balance electronic weighing machine and randomly allotted to three treatments. Prior to offering the experimental dietary treatments, the birds were fed on a common commercial diet and clean drinking water was provided ad libitum. Feed was weighed and provided to the birds on a daily basis. The daily feed ration was offered in two equal parts in the morning and afternoon, at 08:30 h and 14:30 h, respectively. The initial body weight for each replicate was recorded at the beginning of the experiment, and then on a weekly basis to determine changes throughout the experimental period.

2.3 Experimental diets

The three experimental diets were formulated, with fish meal partially substituted with dried goat rumen contents (DGRC) at 0, 5 and 10 % (Table 1). Rumen contents were collected from slaughtered goats at Kampala city abattoir following wet season. They were sundried to 10 % moisture content and milled to particle size of 1.5 mm.

Table 1: Ingredient composition (%) of layer diets, as fed.

	Layer's mash			
Ingredients (%)	Diet 1	Diet 2	Diet 3	
DGRC*	0	5	10	
Broken maize	49	53	51	
Wheat pollard	18.5	13	12	
Fish meal	10	8	6	
Soybean meal	7.5	6	6	
Lake shells	12	12	12	
Dicalium phosphate	2	2	2	
Salt	0.5	0.5	0.5	
Vitamin premix [†]	0.5	0.5	0.5	
Total	100	100	100	
Calculated (%)				
Dry matter	73.73	76.85	79.76	
Crude protein	16.43	16.37	16.44	
Calcium	3.51	3.54	3.50	
Phosphorus	0.78	0.93	1.08	
Crude fibre	3.77	4.328	5.06	
$ME^{\ddagger} (MJ kg^{-1})$	11.24	11.31	11.12	
Cost/kg fresh feed mix (USD)	0.36	0.35	0.34	

*DGRC: dried goat rumen contents. [†] Premix in the diet provided per kilogram; Vitamins; A 1200000 iu; D3 2500000 iu; E 20000 mg; K3 2000 mg; B1 2000 mg; B2 5000 mg; B6 4000 mg; B12 15 mg; Niacin 30000 mg; Pantothenic acid 11000 mg; Folic acid 1500 mg; Biotin 60 mg; Choline chloride 220000 mg; Antioxidant 1250 mg; Mn 50000 mg; Zn 40000 mg; Fe 20000 mg; Cu 3000 mg; I 1000 mg; Se 200 mg; Co 200 mg. footnotemark[3] ME: metabolisable energy; Diet 1; 0 % DGRC; Diet 2: 5 % DGRC; Diet 3: 10 % DGRC. 1 USD = 3700 Ugandan shillings

The diet with 0% DGRC substitution was the control. Each treatment consisted of 10 birds with three replicates in a completely randomized design (CRD). The diets were formulated to meet the requirements for the layer chicken with 17.5% crude protein (CP) and 12.1 MJ metabolisable energy (ME) per kilogram of feed as fed (NRC, 2001). Further, care was exercised to optimize the levels of most essential minerals and the Ca:P ratio in the diets. Samples of DGRC meal were analysed for proximate composition according to AOAC (2005).

2.4 Egg production and sensory evaluation

Eggs were collected three times daily during a 5-month period and recorded per diet. The percentage egg production

was calculated as number of eggs produced divided by the total number of hens per treatment.

% Egg production =

$$\frac{\text{Number of eggs produced per treatment}}{\text{Number of birds available per treatment}} \times 100$$

The egg sensory evaluation was carried out by a trained panel consisting of 30 participants aged between 20 and 35 years. The panel judges were instructed on the process of evaluating parameters like appearance, odour, texture, taste, yolk colour and acceptance of the sensory quality of eggs. The parameters were scored on a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely). Ten eggs from each treatment were boiled for 10 minutes and left to cool at room temperature (25 °C). The eggs were then peeled and cut into quarters on a white plate. Each person was placed in a room alone, to prevent mutual influence of the panellists. Sensory evaluation was carried out after breakfast (10:00 h) so that the sensation of hunger did not interfere with the results.

2.5 Partial budget analysis of using DGRC in layer diets

The partial budget was calculated as the difference between the feed costs incurred during the experimental periods with respect to sale of eggs and off layers. The net return (NR) was calculated by subtracting total variable cost (TVC) from total return (TR). The change in net return (Δ NR) was computed by subtracting change in variable cost (Δ TVC) from change in total return (Δ TR). The marginal rate of return (MRR) quantified the increase in net return associated with each additional unit of expenditure. This was expressed by percentage as follows:

$$MRR \% = \frac{\Delta TR}{\Delta TVC} \times 100$$

The feed costs were calculated based on the market price of each ingredient and percentage of DGRC inclusion. Feed consumed by the birds per treatment was multiplied by the cost per kilogram feed to obtain total feed costs. The cost of DGRC included cost of collection, drying, transportation and milling. The current market prices of eggs and off layers were considered during the experimental period as total return.

2.6 Data analysis

The data was subjected to normality and homogeneity of variance test and then analysed using the GLM procedures of SAS (2010) for a completely randomized design. Model sums of square were partitioned to test linear and quadratic trends (Gomez & Gomez, 1983). Mean separation where significant differences occurred was done using Tukey's test at P < 0.05. The following model was used;

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: *Y* is the dependent variable; μ is the mean; *T* effect of treatment (*i* = 1, 2, 3); ε_{ij} random error term. The effects of DGRC on sensory data was analysed using analysis of variance (ANOVA) at 5% level of significance. Where the means were significant, mean separation was done using turkey's honest significant difference (HSD).

3 Results

3.1 Chemical composition of experimental diets

Chemical composition of the experimental diets is shown in Table 2.

Table 2: Chemical composition of dried goat rumen contents(DGRC) based layer diets.

	Layer's mash			
Parameters	Diet 1	Diet 2	Diet 3	SEM
DM %	88.74	88.03	88.12	0.12
CP %	17.31	17.20	17.07	0.08
EE %	2.50	2.59	3.44	0.01
CF %	4.43	4.46	4.61	0.03
Ash %	17.51	17.15	16.66	0.29
Ca %	4.12	3.95	4.06	0.05
Р%	0.93	0.89	0.93	0.01
NFE %	58.23	60.59	58.22	0.30
ME MJ/kg DM	12.48	12.07	12.64	0.04

DM: dry matter; CP: crude protein; CF: crude fibre; NDF: EE: ether extract; Ca: calcium; P: phosphorous; NFE Nitrogen free extract; ME: metabolisable energy. SEM: standard error of the mean; Diet 1: 0% DGRC; Diet 2: 5% DGRC; Diet 3: 10% DGRC.

Dry matter (DM) and ether extract (EE) concentrations were similar across diets. Crude fibre (CF) concentration in the dietary treatments differed slightly and was higher in the diet with 10 % DGRC inclusion. Despite the slight disparities in the CF contents of the diets, they were within the range (2-5 %) recommended for optimum layer performance by NRC (2001). Metabolisable energy (ME) concentration of the dietary treatments ranged between 11.51 and 12.56 MJ kg-1 feed DM.

3.2 Nutrient digestibility

The digestibility of nutrients in layer diets is shown in Table 3.

	Diet	ary treatn	nents		
Digestibility %	Diet 1	Diet 2	Diet 3	SEM	P-value
Dry matter	73.41 ^b	72.93 ^b	74.12 ^a	1.65	0.0254
Crude protein	64.52	66.89	66.91	0.50	0.0677
Crude fibre	62.10 ^c	72.29^{a}	76.69^{b}	0.79	0.0021
Calcium	68.66^{b}	66.4 ^{ab}	66.91 ^a	1.84	0.0039
Phosphorous	74.29	69.66	69.98	0.95	0.0688
Ether extract	87.27^{b}	69.34 ^a	92.95 ^a	0.14	< 0.001
NFE	97.79 ^b	96.65 ^a	96.70 ^a	0.94	0.0173

 Table 3: Digestibility of nutrients of dried goat rumen contents (DGRC) based layer diets.

^{*abc*} Means within a row with different superscripts differ significantly

(P < 0.05); NFE: Nitrogen free extract; SEM: standard error of mean; Diet

1; 0 % DGRC; Diet 2: 5 % DGRC; Diet 3: 10 % DGRC.

Table 4: Average daily feed intake (ADFI), average daily weight gain (ADG) and
feed conversion ratio (FCR) of layers fed dried goat rumen contents (DGRC) based diets.

	dietary treatments				P-value	
Parameters	Diet 1	Diet 2	Diet 3	SEM	Linear	Quadratic
ADFI (g/bird/day)	159.46 ^a	166.02 ^c	169.35 ^b	0.30	< 0.001	< 0.001
ADG (g/bird/day)	59.73 ^c	51.69 ^b	49.69 ^a	0.28	< 0.001	0.0001
FCR (kg feed/kg weight gain)	2.66 ^a	3.20^{b}	3.40 ^c	0.07	< 0.001	0.0517

^{*abc*} Means within a row with different superscripts differ significantly (P < 0.05); SEM: standard error of mean; Diet 1; 0 % DGRC; Diet 2: 5 % DGRC; Diet 3: 10 % DGRC.

Incorporation of DGRC in the diets at 5 % resulted in significant (P < 0.05) improvement of the digestibility of dry matter (DM) and crude fibre (CF). Inclusion of DGRC had no significant effect (P>0.05) on the digestibility of phosphorus (P), crude protein (CP), and metabolisable energy (ME), but there was a significant (P < 0.05) decrease in calcium digestibility with increasing incorporation of DGRC.

3.3 Effect of DGRC incorporation on daily feed intake, feed conversion ratio and mortality rate

The effects of incorporating DGRC in layer diets on ADFI and FCR are presented in Table 4. Diet significantly (linear and quadratic model P < 0.05) affected ADFI, ADG and FCR. There was an increase in ADFI and FCR with increasing levels of DGRC inclusion.

3.4 Effect of DGRC incorporation on laying percentage and egg sensory characteristics

Figure 1 shows the laying percentage as affected by the inclusion of DGRC in layer diets. The results showed that laying percentage decreased linearly with an increase of DGRC in the diets. However, laying percentage did not differ in layers fed on 0 % and 5 % DGRC diets (P > 0.05).



Fig. 1: Effect of DGRC inclusion in layer diets on laying percentage (including standard deviation). (DGRC: dried goat rumen contents; Diet 1: 0% DGRC; Diet 2: 5% DGRC; Diet 3: 10% DGRC.)

Egg sensory attributes as affected by incorporation of DGRC in layer diets are shown in Table 5. The level of incorporation of DGRC in layer diets did not affect (P > 0.05) egg appearance and odour. However, egg acceptance and yolk colour were significantly (P < 0.05) affected by inclusion of DGRC, with eggs from layers fed 10 % DGRC being more acceptable, followed by those from layers fed 5 % DGRC. Eggs from layers fed on diets with 0 % DGRC incorporation were least accepted by the panellists. A significant ef-

Table 5: Effect of dried goat rumen contents (DGRC) inclusion in layer diets on egg sensory characteristics.

	mean			
Attribute (n=36)	Diet 1	Diet 2	Diet 3	P-value
Appearance	6.80 ± 1.95	7.63 ± 2.05	7.44 ± 2.10	0.1980
Odour	6.11 ± 2.53	6.22 ± 1.48	6.69 ± 2.18	0.0784
Texture	$6.22^a \pm 2.55$	$7.30^b \pm 1.78$	$7.36^b \pm 1.86$	0.0381
Acceptance	$5.33^a \pm 2.65$	$7.80^b \pm 1.67$	$7.91^b \pm 1.61$	< 0.0001
Colour of yolk	$2.52^a \pm 1.95$	$7.25^b \pm 2.04$	$7.77^b \pm 1.77$	< 0.0001

Mean values within row followed by different superscripts are significantly different (P < 0.05) * Mean±SD; Diet 1: 0 % DGRC; Diet 2: 5 % DGRC; Diet 3: 10 % DGRC.

Table 6: Effect of DGRC inclusion in layer diets on egg sensory characteristics.

Variable	Diet 1	Diet 2	Diet 3
Total feed intake (kg as fed)	631.70	589.40	524.10
Total feed cost/treatment* (USD)	204.66	159.39	150.36
Feed cost/kg as fed (USD)	0.32	0.27	0.29
Labour cost for processing DGRC (USD)	0.00	1.22	2.43
Total variable costs (TVC, USD)	204.66	160.61	150.65
Gross income (TR) (eggs + birds sold, USD)	327.13	299.32	266.88
Net Return (NR, USD)	122.47	138.70	116.23
Change in TR (USD)	0.00	16.23	-6.24
Change in TVC (USD)	0.00	44.05	54.01
Change in NR (USD)	0.00	16.23	-6.24
MRR (%)	0.00	36.86	-11.55
ADG (g/bird/day)	37.93	33.81	32.84
Feed cost / kg gain (USD)	5.40	4.71	4.58
TR / feed cost (USD)	1.60	1.88	1.77

Chicken sale =5.41 USD/layer; DGRC= dried goat rumen contents; Diet 1: 0 % DGRC; Diet 2: 5 % DGRC; Diet 3: 10 % DGRC; * each treatment contained a total of 30 birds; TVC = Total Variable Costs; TR = Total Revenue; NR = Net Revenue; MRR = Marginal Rate of Return; ADG = Average Daily Gain. 1 USD = 3700 Uganda Shillings.

fect (P < 0.05) of treatments on colour of yolk was observed, with eggs from 10 % DGRC diets having more dark yellow yolks than eggs from 0 % and 5 % diets.

3.5 Economics of inclusion of DGRC in layer diets

Marginal rate of return (MRR) was higher (36.86%) for layers on 5% DGRC diet. However, as the level of DGRC increased to 10%, MRR also decreased to -11.55%. It was generally observed that incorporation of DGRC in layer diets resulted in a feed cost reduction per kilogram of feed. In comparison to the 0% DGRC diet, the 5% and 10% DGRC diets resulted in 15.62% and 9.37% reduction in feed costs per kilogram of feed, respectively.

4 Discussion

4.1 Chemical composition of the experimental diets

Crude fibre (CF) concentration increased with increasing levels of DGRC inclusion in the diets. Rumen contents have high fibre content and therefore there is a limit to which they can be incorporated in layer diets without compromising performance. This finding was in line with the results of Djordjevic *et al.* (2006). Despite the disparities in the CF content in layers diets, CF levels of the diets were within the range (2-5%) recommended for optimum layer performance by NRC (2001). Protein and metabolisable energy concentrations in the diets were within the range of 16 - 17% CP and 2750 - 3000 kcal ME kg⁻¹ feed, respectively, that elicit proper laying responses (NRC, 2001). Protein and energy are vital feed components for poultry (Dairo *et al.*, 2010), and they play a significant role in the early egg laying phase,

immune response and overall adaptation to the environment (Field *et al.*, 2002; Adedokun & Olojede, 2019). In general, diets for poultry should be formulated with precision in order to me*et al*l nutritional requirements for optimal performance (Le *et al.*, 2012).

4.2 Effect of incorporation of DGRC on nutrient digestibility

Incorporation of DGRC at 5 % level in the layer diets led to improvements in the digestibility of CP, CF and DM. This indicated that there is a limit to which rumen content can be incorporated in layer diets, beyond which digestibility coefficients become compromised. Rumen content also contains good quality protein with a high share of essential amino acids (Esonu *et al.*, 2006; Agbabiaka *et al.*, 2012; Elfaki & Abdelatti, 2015), however, in this study, amino acid composition of the experimental diets was not determined. Improvement of CF digestibility at 5 % DGRC incorporation could point to better gastrointestinal tract health with better microbial colonisation which led to higher fibre digestion (Kheravii *et al.*, 2017).

4.3 Effect of DGRC on feed intake and feed conversion ratio

Since feed account for the highest share of production cost, ADFI of birds is an important economic factor that determines profitability. The increase in ADFI with increasing levels of DGRC in layers diets was in line with the findings of Efrem et al. (2016) and Bekele et al. (2020). As the dietary level of DGRC increased, there was a concomitant increase in CF content that could have led to a decrease in dietary energy density and eventually increased feed intake by the birds in order to meet their energy requirements and sustain egg production (Efrem et al., 2016). However, despite the increase in ADFI with increasing inclusion levels of DGRC, there was low ADG among the birds, especially before attaining the laying stage. This might have been due to interferences in nutrient digestibility, especially concerning dry matter and gross energy (Cozannet et al., 2010). FCR was lower for birds fed 0 % DGRC compared to those fed on 5 % and 10 % DGRC, despite the reduced feed cost with use of DGRC. This could be probably attributed to a lower crude fibre content in 0 % DGRC diet compared to 5 % and 10 % DGRC diets which could have led to better nutrient digestibility and utilisation by the birds (Yokhana et al., 2016).

4.4 Effect of DGRC on laying percentage and egg sensory characteristics

Inclusion of DGRC in layer diets led to a decrease in laying percentage which might have been the result of nutrient dilution by adding DGRC or due to nutrient imbalances. This result concurs with the findings of Odunsi (2003) and Bekele *et al.* (2020) who reported decrease in egg production as the levels of rumen content increased in the diets. Moreover, despite the increase in ADFI with increasing levels of dietary DGRC, the hens' ADG was low which could have resulted in subsequent low production performance and a decrease in laying percentage.

Use of dried goat rumen contents in layers' diets did not affect appearance, odour and texture of eggs which was in agreement with the findings of Bekele et al. (2020). There was, however, an increase in yolk colour intensity with increasing inclusion of DGRC, which concurred with the findings of Efrem et al. (2016). This colour might be a result of increased xanthophyll content in the diets due to rumen content incorporation. Xanthophyll, the oxygen derivative of carotenoids, is contained in the form of carotenoids in green forages consumed by herbivores (Prache et al., 2003). Xanthophyll in the rumen content was responsible for egg yolk pigmentation, which in turn depended on digestibility, metabolism, transfer and deposition of carotenoid within the yolk (Titcomb et al., 2019). Figure 2 shows the yolk colour changes in fresh and boiled eggs as a result of rumen content inclusion in the layer diets.



Fig. 2: Fresh egg yolks (left) and boiled eggs (right) from layers fed with 0 % DGRC (a), 5 % DGRC (b) and 10 % DGRC (c) diets. (Photo credit: Robert Mwesigwa).

Yolk colour intensity increase with increasing DGRC in the diets contributed to the increased acceptance of the eggs by the panellists. When investigating synthetic carotenoid effect on yolk colour, Englmaierová *et al.* (2013) reported that the choice of consumers for eggs is not only based on internal quality characteristics like cholesterol level and fatty acid profile but also on yolk colour. Hens do not have the capacity to synthesize carotenoids and for yolk colour pigmentation, carotenoids must therefore be supplied as dietary ingredients (Karadas *et al.*, 2006; Bouvarel *et al.*, 2011). The 10% DGRC incorporation in the diets apparently led to increased levels of carotenoids which were digested and deposited in the egg, leading to a more intense dark yellow yolk as compared to the diets with 0% and 5% DGRC. Inclusion of DGRC in diets also led to an increased acceptance of eggs which was contrary to the findings of Bekele *et al.* (2020) who reported no difference in egg acceptance with use of rumen contents in diets of laying hens. This could have been due to a perceived better quality of the eggs with deep yellow yolk colour. Consumers are increasingly becoming aware of food quality and get more attracted by better quality eggs with firm albumen and deep yolk colour (Samiullah *et al.*, 2014).

4.5 Economics of including DGRC in layer diets

The reduced cost of feed as a result of incorporation of DGRC in layer diets was due to price differences of fish meal and DGRC (Uchegbu *et al.*, 2006). In this study, the cost per kilogram of DGRC (inclusive of collection, drying, transportation and milling) was estimated at USD 0.081 whereas fish meal was bought at USD 0.81. Higher economic returns (marginal rate of return) were realised at 5 % DGRC inclusion, beyond which economic returns declined in agreement with the findings of Sugiharto *et al.* (2019) who reported reduced feed costs with use of alternative non convention protein sources like leaf meals. The current results are not only crucial for poultry businesses but also to the abattoir operators who have been grappling with rumen content disposal challenges (Aniebo *et al.*, 2009).

5 Conclusion

It was concluded that incorporation of DGRC at 10% in layer diets improved egg yolk colour and acceptability of the eggs. Economically, only an inclusion of DGRC at 5% in layer diets led to a decrease in feed costs and improved marginal rate of return, mainly due to reduced laying performance of the birds.

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Ethical standards

This research followed the ethical guidelines of the Uganda National Council of Science and Technology.

Conflict of interest

The authors declare no conflict of interest.

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135

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