

# Impact of Replacing Soybean Meal with Sunflower Meal, Sesame Meal, and Black Seed Meal in diets of Barki Lambs

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## ABSTRACT

Crude protein content in animal diet is considered the first important factor for nutritionists when they start to formulate a diet for different types of animals. Therefore, this experiment was conducted to investigate the effect of substituting sovbean seed meal by different protein sources on lamb's diet in terms of nutrients digestibility, and growth performance. Therefore, four tested concentrate feed mixtures (CFM, 14% crude protein) were designed. The CFM1 contained soybean meal as the only source of protein, and 50% of soybean meal protein was replaced by either sunflower meal, sesame seed meal, or black seed meal in CFM2, CFM3, and CFM4 groups, respectively. A total of 28 growing Barki male lambs aged 6 months with an average body weight of  $38.6 \pm 0.4$  Kg were used in feeding trials for 120 days. Depending on their body weight, lambs were divided into four groups (7 animals in each). Each group fed on one of the experimental diets, including D1 (CFM1+ clover hay), D2 (CFM2 + clover hay), D3 (CFM3 + clover hay), and D4 (CFM4 + clover hay). Results of digestion coefficients indicted that the digestibility of crude protein and the nitrogen-free extract was significantly higher for D1 and D4, compared to D2 and D3. The same trend was observed in nutritive values expressed as total digestible nutrients (TDN) and digestible crude protein (DCP), whereas the D1 and D4 recorded higher TDN and DCP, compared to D2 and D3. Results of rumen parameters demonstrated that there were insignificant differences among groups regarding the average pH, ammonia (NH3-N), and total volatile fatty acids (TVFAs). Data of growth performance indicated that the final live body weight, total body gain, and average daily gain of lambs feed D4 (56.75 kg, 18.62 kg, and 155 g, respectively) and D1 (56.62 kg, 17.60 kg, and 147 g, respectively) were higher than those for lambs fed D2 (53.40 Kg, 14.64 kg, and 122 g, respectively) and D3 (53.11 kg, 14.61 kg, and 122 g, respectively). The feed conversion ratio (feed/gain) was recorded better value with lambs fed D4 followed by that fed D1. In conclusion, black seed meal could be replaced with the 50% of soybean meal participation of protein in growing Barki lambs' diets without any adverse effects on digestibility, rumen kinetics, and growth performance.

Keywords: Barki lambs, Black seed meal, Digestibility, Growth, Sesame meal, Sunflower seed meal

# INTRODUCTION

Feed plays a significant role in animal production progress and accounts for 60-70 percent of total livestock production expenses in any given year (Abo Omar, 1998; Ghorbani et al., 2018). The main two nutrients in animal nutritional requirements are protein and energy. Therefore, nutritionists always search for alternative sources of energy and protein to reduce the cost of the feeding budget. At the same time, ruminants could maximize utilization of roughages by fermentation process in the rumen to produce volatile fatty acids and it is considered a main source of energy in rumen blood. Proteins are a significant nutrient for every tissue and have a vital role for all body organs. For example, antibodies, some hormones, and catalysts are totally or partially considered as units of protein (Suliman and Babiker, 2007).

Plant-based feeds are the main source of protein in animals' diets to meet their amino acid requirements and other biological processes in the body (Elliot, 1980). Therefore, there is a need to conduct research on alternative sources of protein to cover demands of protein in animal diets, especially with price changes and frequent accessibility issues of the feedstuff market (Lawrence et al., 2008; Stillman et al., 2009).

Some plant-based feeds are a by-product of oilseeds after oil extraction. Based on the method of oil extraction (mechanical or solvent extraction) the chemical composition of meals may vary (Ministry of Agriculture, 2007).

Soybean meal (SBM) is the main and traditional source of protein in animal diets (Schingoe et al., 1979; Belal et al., 2019b). Nowadays, there are alternative oil by-products, such as sunflower, sesame, and black seeds Nigella sativa, that could be used as a source of protein and energy in ruminants' diets after oil extraction.

Sunflower seed meal (SFM) is a good source for feeding sheep diet in a growth period because it contains oil with a high level of polyunsaturated fatty acids (65.3%), and crude protein (CP, 22-33%, Andy et al., 2011; Nagalakshmi et al.,

ORIGINAL ARTICLE pii: S232245682100084-11 Received: 28 October 2021 Accepted: 12 December 2021 2011). Economides and Koumas (1999) showed that sunflower seed meal could effectively replace soybean seed meal in sheep diet of optimal growth diet with no adverse effects on health or production.

Sesame hulls entail about 50% oil and 20-25% CP (Hirano et al., 2002; Obeidat et al., 2009). Moreover, the use of sesame seed meal (SSM) at 10% and 20% has a positive effect on growth performance and decreases feeding costs of the diet of growing lambs (Abo Omar, 2002).

Black seed meal (BSM) with the botanical name of Nigella sativa is considered as one of the unconventional feed protein sources. Black seed has vital therapeutic properties and is usually consumed as medicinal oil. The oil residue of the Nigella sativa meal is also high in fatty acid (Oleic, Linolenic, and Linoleic acid) and carotene, which is turned into vitamin A (Al-Jassir, 1992). Black seed meal contains most of the essential amino acids, and 33% CP (Zaky et al., 2021). Moreover, it includes some specific phytochemicals (tannins, trypsin inhibitors, flavonoids, saponins) that might affect positively digestion, and consequently, improve the production performances of animals (Makkar et al., 2007). Therefore, the current study aimed to investigate the effect of partial replacement of soybean meal as a conventional source of protein in the diet of Barki lambs by SFM, SSM, and BSM as alternative sources of protein diet on nutrient digestibility, rumen parameters, and growth performance.

# MATERIALS AND METHODS

## Location and Ethical consideration

This study was completed at Agricultural Experimental Station, Sheep and Goat Research Unit, Faculty of Agriculture, Cairo University, Egypt. The study occurred under ethical consideration of the Institutional Animal Care and Use Committee (IACUC) of Cairo University under application number CU/II/F/24/2.

#### **Experimental concentrate feed mixtures**

Four experimental concentrate feed mixtures were formulated to contain SBM as the only source of protein or mixed with either SFM, SSM, or BSM (Table 1). Concentrate feed mixtures (CFMs) formulation which contained SBM as CFM1, while SFM, SSM, or BSM in the other experimental concentrate feed mixtures named CFM2, CFM3, and CFM4 replaced as 50% of soybean meal protein, respectively (Table 2).

Tested feeds	Classes have	CDM	CEM	CCM	DCM
Chemical compositions (%)	Clover hay	SBM	SFM	SSM	BSM
Dry matter	93.01	89.18	92.7	89.49	88.96
Organic matter	85.95	88.96	92.9	89.8	91.57
Ash	14.05	11.04	7.1	10.2	8.43
Crude protein	11.47	44.21	31.2	32.35	33.13
Ether extract	2	2.27	1.5	15.45	12.72
Crude fiber	26.27	6.67	10.22	9	10.96
Nitrogen free extract	46.21	35.81	49.98	33	34.76

Table 1. Chemical composition of clover hay and different protein sources in the experiment on a dry matter basis

SBM: Soybean meal, SFM: Sunflower meal, SSM: Sesame meal, BSM: Black seed meal

<b>Table 2.</b> Formulation of the exp	perimental concentrate feed	l mixtures on a dry	matter basis

	The experimental concentrate feed mixtures (for 120 days)						
Ingredients (%)	CFM1	CFM2	CFM3	CFM4	CFM1		
Corn	65	64	63	63	65		
Soybean meal	15	7.5	7.5	7.50	15		
Sunflower seed meal		9.1					
Sesame meal			10.4				
Black seed meal				10			
Wheat bran	18	17.4	17.1	17.5	18		
Vitamins and minerals premix	0.3	0.3	0.3	0.3	0.3		
Common salt	0.7	0.7	0.7	0.7	0.7		
Calcium carbonate	1	1	1	1	1		
Total	100	100	100	100	100		

CFM1: Concentrate feed mixture 1, including soybean meal, as the only source of protein; CFM2: Concentrate feed mixture 2, including Soybean meal + sunflower meal; CFM3: Concentrate feed mixture 3, including Soybean meal + sesame seed meal; CFM4: Concentrate feed mixture 4, including Soybean meal + black seed meal

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## Animal management, experimental diet, and feeding procedures

A total of 28 growing male lambs (Barki breed) with an average body weight of  $38.6 \pm 0.4$  Kg, aged 6 months were kept at the experimental research station of Faculty of Agriculture, Cairo University, Egypt, under veterinary medical care (towards internal and external parasites) for a preliminary period of 15 days. At the end of the preliminary period, lambs were divided based on their body weight into four groups of seven for 120 days growth trial. Each group of animals fed on one of the experimental diets according to NRC (1985) to cover the nutritional requirements of growing lambs in this stage of life. The experimental diets included D1 as CFM1 + Clover hay, D2 as CFM2 + Clover hay, D3 as CFM3 + Clover hay, and D4 as CFM4 + Clover hay. Each group was kept in a separated brick-made pen. The animals were weighed every two weeks during the growth trial before feeding after 17 hours of fasting. The animals were offered their diet twice daily at 8 am and 5 pm. Amounts of diet offered were adjusted every two weeks to ensure that the rations covered the requirements of the animals. Water and salt blocks were available *ad labium* to the animals. The remaining of the feeding of the previous day were weighed consistently before the feeding of a new day. Meanwhile, feed intake was recorded daily then daily body weight gain and feed conversion (feed intake/gain) were calculated.

## **Digestibility trials**

In this phase of the study, of the 28 lambs in the feeding trial, 12 were assigned into four groups in digestion trial (3 lambs from each group) for 21 days, including14 days as a starter period and 7 days as a collection period, to evaluate the digestion and nutritive value of the tested diets (75% CFM + 25% hay). Animals were kept in metabolic confines and fed their diets, which provide the maintenance requirements, twice a day in equal amounts of concentrate feed mixtures and clover hay at 8.00 am and 5.00 pm (Chagas et al., 2015) and water was freely accessible. During the collection period, feces were collected daily then dried directly and kept for chemical analysis (Khattab et al., 2011). Nutritive values as total digestible nutrients calculated according to McDonald et al. (1995), as TDN (%) = % digestible of CP + % digestible of CF + % digestible of nitrogen-free extract (NFE) + (% digestible of EE \*2.25).

#### **Rumen liquor parameters**

Toward the end of the digestion trials, the rumen liquor samples were taken from the experiment animals via a stomach tube (Muizelaar et al., 2020). The rumen liquor samples were taken before morning feeding at 7.30 am then at 3 and 6 hours post-feeding as described by Khattab et al. (2011). The samples were sifted through four layers of surgical gauze to get clear rumen liquid. Ruminal pH was immediately recorded using a digital pH meter, then the samples were kept ( $-5^{\circ}C$ ) to determine rumen ammonia and total volatile fatty acids (Hofirek and Haas, 2001).

#### **Chemical analysis**

Samples of ingredients and complete diets, orts, and feces were analyzed for moisture, CP, ether extract, crude fiber, and ash, according to AOAC (2002). Accordingly, the calculation of nitrogen-free extract was measured as %NFE = 100 – (% secondary moisture + % ash + % CP + % EE + % CF) based on the differences between soluble and insoluble material components.

## **Rumen liquor parameters**

The ruminal pH was measured immediately after rumen liquor collection using the HANNA pH meter, model (Hanna HI8424 Portable pH/mV Meter, Waterproof). The concentration of ruminal ammonia nitrogen (NH<sub>3</sub>-N) was determined by applying the NH<sub>3</sub> diffusion technique, Kjeldahle distillation method according to AOAC (2002). Total volatile fatty acids (TVFA's) concentration was determined by steam distillation as suggested by Kromann et al. (1967).

#### Statistical analysis

Data obtained from the present study were statistically analyzed using SAS (2000). One-way ANOVA procedure was used to evaluate the data using  $Y_{ij} = \mu + D_{ij} + E_{ij}$ ; where,  $\mu$  is the overall mean of  $Y_{ij}$ ,  $D_{ij}$  signifies the effect of the diet, and  $E_{ij}$  denotes the error during trial. Differences in means among groups were compared by Duncan's Multiple Range Test (Duncan, 1955) based on a significance level of 0.05.

# **RESULTS AND DISCUSSION**

#### **Chemical composition**

Data in tables 1, 2, 3, and 4 explained the chemical composition of the experimental meals, the formulation, the chemical composition of the experimental concentrate feed mixtures, and the experimental diets, respectively. The chemical composition SBM, SFM, SSM, and BSM is similar to the findings of FAO (1990), Ayman and Abo Omar (2009), Mulugeta and Gebrehiwot (2013), Mahmoud and Bendary (2014), and Hamed et al. (2019). The experimental concentrate feed mixtures were almost similar in their chemical composition. However, CFM1 recorded the highest CP content followed by concentrate CFM4, CFM3, and CFM2 as 16.01%, 15.98%, 15.85%, and 15.51%, respectively. The

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highest ether extract content was recorded for CFM3 (3.61%) followed by CFM4 (3.27%), CFM1 (2.17%), and CFM2 (2.14%). A similar pattern was also observed for ash content. This chemical composition of the experimental concentrate feed mixture was reflected in the chemical composition of experimental diets (Table 3). These results agreed with the findings of Awadalla (1997), Irshaid et al. (2003), Ayman and Abo Omar (2009), and Mahmoud and Ghoneem (2014).

Table 3. The chemical	composition of th	ne experimental	concentrate feed	s mixtures on a dry matter basis

	The experimental concentrate feed mixtures (for 120 days)						
Chemical composition (%)	CFM1	CFM2	CFM3	CFM4			
Organic matter	95.51	95.69	95.28	95.5			
Ash	4.49	4.31	4.72	4.5			
Crude protein	16.01	15.51	15.85	15.98			
Ether extract	2.17	2.14	3.61	3.27			
Crude fiber	4.56	5	5	5.16			
Nitrogen free extract	72.77	73.04	70.82	71.09			

CFM1: Concentrate feed mixture 1, including soybean meal, as a lone source of protein; CFM2: Concentrate feed mixture 2, including soybean meal + sunflower meal; CFM3: Concentrate feed mixture 3, including Soybean meal + sesame seed meal; CFM4: Concentrate feed mixture 4, including Soybean meal+ black seed meal.

	Table 4. Chemical	compositions of th	e experimental diet	s on a dry matter basis
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	The experimental diets					
Chemical composition (%)	D1	D2	D3	D4		
Organic matter	93.12	93.26	92.95	93.11		
Ash	6.88	6.74	7.05	6.89		
Crude protein	14.88	14.5	14.76	14.85		
Ether extract	2.13	2.11	3.21	2.95		
Crude fiber	9.99	10.32	10.32	10.44		
Nitrogen free extract	66.12	66.33	64.66	64.87		

D1: Concentrate feed mixture 1 + Clover hay, D2: Concentrate feed mixture 2 + Clover hay, D3: Concentrate feed mixture 3 + Clover hay, D4: Concentrate feed mixture 4 + Clover hay

## Nutrients digestibility and nutritive values

Results in Table 5 showed insignificant differences among experimental diets for dry matter, organic matter, ether extract, and crude fiber (p > 0.05). Moreover, there was an insignificant difference (p > 0.05) between D1 and D4 in the digestibility of CP (70.32% and 71.67%) and NFE (78.82% and 79.80%, respectively). However, there was a significant decrease in the digestibility of CP and NFE by 6% and 3.8% for D2 and by 7% and 5.2% for D3 compared with D1 (p < 0.05). These results might be due to the similarity in the chemical composition between D1 and D4 and between D2 and D3 as mentioned by Mahmoud and Bendary (2014). This result agreed with the findings of Irshaid et al. (2003) and Mahmoud et al. (2017) that nutrient digestibility for SBM diets fed to growing calves was more significant than the SFM diet. The results agreed with the findings of Mahmoud and Ghoneem (2014) that the substitution of SBM and cotton meal with sesame meal and grass in growing lambs decreased the nutrients digestibility. However, these results disagreed with the findings of Belal et al. (2019a) that replacing soybean meal with sesame meal in Awassi sheep diets showed no statistically significant differences in nutrient digestion. The results of nutrient digestibility were reflected on the nutritive value, whereas there were insignificant (p > 0.05) differences in total digestible nutrients (TDN, 72.47% and 11.46%) and digestible crude protein (DCP, 73.92% and 10.64%) between D1 and D4, respectively. On the other hand, TDN and DCP for D2 and D3 were significantly decreased by (3.8 % and 16.5 %) and (3.9% and 15.8%), respectively (P < 0.05).

# **Rumen liquor parameters**

Regarding the sampling time, as indicated in Table 6, the values of pH before feeding were high, then reduced after 3 hours from feeding, then increased at 6 hours after feeding for all tested diets. This result was caused by the sturdy fermentation process of both nonstructural and structural carbohydrates and the production of volatile fatty acids. This result agreed with the findings of Sabbah et al. (2011). There was an insignificant difference through groups in the average pH being, 7.46, 7.13, 7.26, and 7.31, respectively for D1, D2, D3, and D4 (p > 0.05). On the contrary, concentdiets of rumen ammonia (NH3-N) and total volatile fatty acids values (TVFAs) were low before feeding, then increased at 3 hours post-feeding, and decrease at 6 hours post-feeding for all experimental diets. There were no significant differences among groups. These results agreed with results obtained by Ahmed and Abdalla (2005), Moura et al. (2015), Ghorbani et al. (2018), and Hamed et al. (2019).

# Feed intake and growth performance

Data of growth performance of lambs fed experimental diets are given in Table 7. It indicated that there were no significant (p > 0.05) differences among groups in initial body weight, final body weight, and total gain. Moreover, there was an insignificant (p > 0.05) difference between lambs fed either D1 or D4 in the daily gain being, 147 and 155 g, respectively. However, feeding lambs either D2 or D3 significantly (p < 0.05) decreased the daily gain by the same trend (17%), compared to those fed D1. This means that lambs fed either D1 or D4 (0.076 and 0.077 kg) had the highest total digestible nutrients (TDN) content per one-kilogram metabolic body weight ( $W^{0.75}$ ), compared to those fed either D2 or D3 (0.072 kg TDN/ Kg  $W^{0.75}$ ) due to the variation among groups in TDN content. These results agreed with the finding of Mahmoud and Bendary (2014) and Abd El-Rahman et al. (2011). Almost, dry matter intake was similar in all groups and ranged from 1.830 to 1.900 Kg/head/day. The best feed conversion ratio was recorded in lambs fed D4 (12.26 DMI/g gain and 9.03 TDN/g gain) followed by those fed D1 (12.92 DMI/g gain and 9.39 TDN/g gain), these results may be due to better average daily gain in groups D4 and D1, compared to the other two groups (D2 and D3).

	The experimental diets					
Parameters	D1	D2	D3	D4	±SE	
Digestion coefficients (%)						
Dry matter	74.62	73.47	73.03	73.6	2.05	
Organic matter	76.62	75.47	75.03	75.6	2.08	
Crude protein	70.32 <sup>a</sup>	66.03 <sup>b</sup>	65.36 <sup>b</sup>	71.67 <sup>a</sup>	1.46	
Ether extract	76.16	75.21	73.65	75.6	1.63	
Crude fiber	62.4	60.67	61.34	62.18	1.18	
Nitrogen free extract	$78.82^{a}$	75.83 <sup>b</sup>	74.73 <sup>b</sup>	$79.80^{a}$	0.73	
Nutritive value (%)						
Total digestible nutrients	72.47 <sup>a</sup>	69.7 <sup>b</sup>	69.62 <sup>b</sup>	73.92 <sup>a</sup>	0.73	
Digestible crude protein	$11.46^{a}$	$9.57^{b}$	9.65 <sup>b</sup>	10.64 <sup>a</sup>	0.41	

 $a_{i}$  and b means in the same row with different superscripts are significantly different (p < 0.05). D1: Concentrate feed mixture 1 + Clover hay, D2: Concentrate feed mixture 2 + Clover hay, D3: Concentrate feed mixture 3 + Clover hay, D4: Concentrate feed mixture 4 + Clover hay, ±SE: standard error.

Table 6.	Effect of	f feeding e	experimental	diets on rumen	liquor	parameters of Barki sheep	

Dumon nonomotors	Compling time		The experimental diets				
Rumen parameters	Sampling time	D1	D2	D3	D4		
	Just before feeding	7.6	7.2	7.37	7.33		
-II	3 hours post-feeding	7	6.77	6.73	6.87		
рН	6 hours post-feeding	7.77	7.43	7.67	7.73		
	Mean	7.46	7.13	7.26	7.31		
	Just before feeding	40.31	37.61	40.49	41.8		
NH <sub>3</sub> -N,	3 hours post-feeding	47.9	46.41	45.83	45.47		
Mg/100 (mlRL)	6 hours post-feeding	42.93	39.2	41.67	41.93		
	Mean	43.71	41.07	42.66	43.07		
Total volatile fatty acids meq/100 (mlRL)	Just before feeding	5.6	5.67	5.2	4.93		
	3 hours post-feeding	7.97	7.27	7.73	7.8		
	6 hours post-feeding	7.17	6.47	6.13	6.60		
	Mean	6.91	6.47	6.35	6.44		

D1: Concentrate feed mixture 1 (CFM1) + Clover hay, D2: Concentrate feed mixture 2 (CFM2) + Clover hay, D3: Concentrate feed mixture 3 (CFM3) + Clover hay, D4: Concentrate feed mixture 4 (CFM4) + Clover hay.

 Table 7. Effects of the experimental diets on live body weight gain, feed intake, and feed conversion of growing Barki lambs for 120 days

		Experimental diets				
Growth parameters of Barki lambs	D1	D2	D3	D4	$\pm$ SE	
Live body weight						
Initial live body weight (Kg)	39.02	38.76	38.5	38.13	2.87	
Final live body weight (Kg)	56.62	53.4	53.11	56.75	3.45	
Total body gain (kg)	17.60	14.64	14.61	18.62	2.8	
Daily gain (g)	147 <sup>a</sup>	122 <sup>b</sup>	122 <sup>b</sup>	155 <sup>a</sup>	11	
Feed intake						
Concentrate DMI, (Kg/head/day)	1.43	1.38	1.37	1.43		
Roughage DMI, (Kg/head/day)	0.47	0.46	0.46	0.47		
Total DMI, (Kg/head/day)	1.9	1.84	1.83	1.9		
TDN intake, (g/head/day)	1.38	1.28	1.27	1.40		
TDN (Kg/Kg *W <sup>0.75</sup> )	0.076	0.072	0.072	0.077		
Feed conversion						
DMI/daily gain (g/g)	12.92	15.08	15	12.26		
TDN intake/daily gain (g /g)	9.39	10.49	10.41	9.03		

<sup>a and b</sup> mean in the same row with different superscripts are significantly different (p < 0.05). D1: Concentrate feed mixture 1 + Clover hay, D2: Concentrate feed mixture 2 + Clover hay, D3: Concentrate feed mixture 3 + Clover hay, D4: Concentrate feed mixture 4 + Clover hay. DMI: Dry matter intake, TDN: Total digestible nutrients, \*W<sup>0.75</sup>: Metabolic body weight, ± SE: Standard error.

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## CONCLUSION

It could be concluded that replacing 50% of soybean meal protein with the same portion of black seed meal protein in the growing lambs' diet had achieved the same results of growth performance of the control diet without any adverse effects on rumen parameters and nutrient digestibility. At the same time using sunflower and sesame seed meals reduced the growth rate by 17% compared to the control diet, so the usage of these two sources depends on the availability of soybean meal and cost of formula when sesame meal and sunflower meal are included in it.

# DECLARATIONS

### **Competing interests**

All authors have participated in conception and design or analysis interpretation of the data, drafting the article or revising it critically for important intellectual content, and approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript. The following authors have affiliations with organizations with a direct or indirect financial interest in the subject matter discussed in the subject matter discussed in the manuscript.

## Authors' contributions

Hesham Mohamed Abd EL- Gawad El-Banna suggested and put the idea, field trials, data analysis, prepared the manuscript writing. Ali Mohamed Ali suggested and put forward the idea, Data revision, prepared the manuscript writing, Reham Roshdy Ali El-Tanany suggested and did data revision, prepared the manuscript writing, and finished the paper. Abderrahim Belkasim Ali Chiab suggested and put the idea, data analysis, field trials, prepared the manuscript writing, and finished the paper. Adel Eid Mohamed Mahmoud revised data and prepared manuscript writing and finished the paper.

## **Ethical considerations**

This study is a practical part of the Master of Science thesis in Animal production of Mr. Abderrahim Belkasim Ali Chiab. This work was designed and implemented totally by authors to participate in feed shortage problem-solving. The field trials were carried out in faculty agriculture, Cairo University in Egypt. The results in the manuscript have been written based on data obtained during trials and chemical analysis. This manuscript was sent only to the World's Veterinary Journal for the publishing process based on the acceptance of all authors. Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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