Nutrient metabolism in ranging tropical dairy cows supplemented with multi-nutrient blocks with varying inclusion of Moringa stenopetala leaves

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Abstract

The formulation of multi-nutrient blocks (MNB) based on low-cost and locally available browse feed resources can be a valid feeding strategy in Sub-Saharan Africa, where inadequate feed supply, both in quality and quantity, is a major constraint. We evaluated the four different inclusion percentage (M-0%, M-25%, M-35%, and M-45%) of *Moringa stenopetala* leaf powder to multi-nutrient blocks on their change on blood metabolite of dairy cows under practical, ranging conditions. Multi-nutrient blocks with four inclusion rates of *M. stenopetala* leaves were applied as complementary feed for free ranging dairy cows. The study was performed on 24 free ranging dairy cows reared around Arba Minch town in the Southern Ethiopian Rift Valley. Blood samples were collected from the jugular vein of dairy cows both before and after supplementation. Plasma glucose, beta hydroxy butyrate (BHB), urea, creatinine, triglycerides, and non-esterified fatty acids (NEFA) concentration was quantified spectrophotometrically. Dried serum spots were subject to quantitative electrospray tandem mass spectrometry to estimate changes in nutrient metabolism based on selected carnitines. Based on these measurements, the milk yield and body condition score were increased during the period of multi-nutrient block supplementation. During the supplementation period, the cows got higher plasma glucose, triglyceride, and urea concentrations and lower concentrations of BHB, NEFA, and creatinine. From the metabolite profiles, a more efficient nutrient use could be concluded. Although no clear dose-response relationship was observed, the highest inclusion of the *M. stenopetala* leaves in the multi-nutrient blocks gave the best performance. This outcome supports the idea of implementing *M. stenopetala* based multi-nutrient blocks on tropical smallholder farms that are not easily accessible to conventional extension services.

1. Introduction

In many southern regions of the world, the production of ruminant livestock is severely hampered by an inadequate availability of feed, both in terms of quality and quantity (De Leeuw 1995; IFAD 2008; FAO 2014). Ruminants' primary sources of food in Sub-Saharan Africa are grazing pastures, crop byproducts, roadsides, crop residues, and crop aftermath (Leng 1991; Alemayehu 2006; Arigbede et al. 2011). Although most essential nutrients, such as fermentable organic matter, digestible by-pass protein, minerals, and vitamins, which are necessary for increased rumen microbial fermentation and improved performance in the host animal, are typically absent from the grasses, crop residues, and agro-industrial by-products during the prolonged dry season (Osuji et al. 1995). These feed resources (grazing pastures and grasses, crop residues, crop aftermath, and roadsides) often contain less metabolizable energy and are less useful for production and productivity (Zinash et al. 1995; McDonald et al. 2002). Animals fed on these feed resources resultantly suffer weight loss, lower birth weight, lower resistance to diseases, and, invariably, reduced reproductive and productive performance (Babayemi and Bamikole 2004; Thornton 2010). The principles underlying the efficient use of fibrous crop residues and standing hay by large ruminants are now well understood.

According to Moss (1994), the activity of microbes is what causes digestion in the rumen. These bacteria need ATP for energy, N (in the form of ammonia, peptides, or amino acids), minerals, and the right pH. To maximise rumen microbial growth, dietary modifications are needed since low-quality forages, like cereal straws, do not contain enough N, fermentable carbohydrates or energy, or minerals to meet microbial needs. Supplementing with N and minerals in the form of mineral blocks made of urea and molasses is one of the most effective strategies to increase the digestion of forages of low quality (Garg and Gupta 1993). In intensive production systems, adding concentrate combinations to the feed—such as cereal grains, cereal bran, or cakes made from oilseeds—has raised intakes and enhanced animal performance. Unfortunately, these supplements are often not fed due to their unavailability and their high costs in tropical range conditions, thus making them too expensive for small-scale farmers (FAO 2014; Makkar et al. 2016).

Instead, the formulation of MNB based on low-cost and locally available browse feed resources can be a valid strategy. Such a feeding strategy is aimed at creating an efficient microbial ecosystem in the rumen and balancing the products of

the fermentative digestion with by-pass nutrients to optimize the use of the available energy and nutrients. MNB has been developed by various researchers (George 1986; Mwendia and Khasatsili 1990).

The leaves of the *M. stenopetala* tree have been successfully used as a locally available protein-rich feed resource for tropical livestock. The promotion of their use as feed resources can reduce overgrazing pressure and soil erosion in sensitive tropical areas (Morales 2000). A study with MNB based on *M. stenopetala* showed increased digestibility of fibrous feeds by up to 20% and increased feed intake by 25 to 30% (ESGPIP 2007). Such MNB can easily be made and used by small-holder farmers and local commercial producers. The technology is particularly interesting in areas where ruminants mainly feed on fibrous crop residues or poor-quality forage diets. Dietary inclusion and evaluation of a new feed ingredient such as *M. stenopetala* in home-based MNB could alleviate the nutritional imbalances in free-ranging dairy under tropical conditions.

Evaluating the impact of *M. stenopetala* MNB on the nutritional status of dairy cows under practical ranging circumstances is difficult because of the obvious lack of controlled circumstances and the lack of suitable tools to measure milk yield and body condition score in tropical field situations. In a previous study (Ketema et al. 2021), a method was developed to monitor the nutritional status of ranging cattle through blood metabolite profiling. This method will here be applied to evaluate the use of four MNB formulae with different inclusion rates of *M. stenopetala* leaves as complementary feed for ranging dairy cows in Arba Minch, Ethiopia.

2. Material and Methods

2.1 Study area and multi-nutrient block (MNB) production

This experiment was conducted in Arba Minch town, which is 436 km (6.065267°N 37.560156°E) south of Addis Ababa, Ethiopia. The climate is characterized as semi-humid tropical with bimodal heavy rainfall, ranging from 800 to 1000 mm per year. The twenty-year mean annual minimum and maximum temperature of the area were 15°C and 38°C, respectively. The leaves of *M. stenopetala* were harvested from the Arba Minch University Biodiversity Research Farm. The leaves were harvested from about 50 randomly selected trees in three sub-locations with similar climatic and soil characteristics in the study area in the middle of the rainy season. The young leaves were collected from *M. stenopetala* orchard trees with an age of about 5 years. The harvests were then pooled, put in fiber sacs, and transported to the Arba Minch University College of Agricultural Sciences cattle research facility within 50 minutes. After arrival, the fresh leaves were spread on a plastic sheet and dried under shade for 10 days. After drying (> 85% DM), leaves were taken to the laboratory, milled through a 5 mm hammer mill sieve, and then stored in large, perforated bags made of nylon fibres. The other feed ingredients were purchased from an Arba Minch livestock feed processing plant, local markets, and other feed distributors in Addis Ababa, Ethiopia.

Four different multi-nutrient block formulae were produced (Table 1) using wheat bran, noug cake, teff straw meal, lime powder, urea, iodized salt, molasses, and cement as the binder with different inclusions of *M. stenopetala* powder (M-0%, M-25%, M-35%, and M-45%) (Table 1). The manufacturing technique of MNB followed the procedures of FAO (2007).

Table 1
Ingredient composition of *M. stenopetala* leaf-based multi-nutrient block formulae. The ratio of water: cement was 2:5 (weight basis).

	Treatments, % by weight (fresh basis)			
Ingredients	**M = 0%**	**M = 25%**	**M = 35%**	**M = 45%**
Wheat bran	30	25	20	15
M. stenopetala	0	25	35	45
Cement	6	6	6	6
Molasses	15	15	10	6
*Noug*** cake	10	10	10	10
Lime powder	7	7	7	7
Tef* straw	25	5	5	4
Urea	5	5	5	5
Common salt	2	2	2	2
Total, %	100	100	100	100
* Tef: *Eragrostis teff*; **noug: *Guizotia abyssinica*				

Molders were made of rectangular wood. Once the ingredient mixture was set in the mold, it was pressed by hand. Then the frame was removed to allow block drying (Ben Salem et al. 2003; FAO 2007). A plastic sheet placed inside the mold prevented the block from sticking to the wall and allowed easy removal from the mold. Once the mixture was placed in the mold and formed by pressure, it was left in a well-ventilated room to set. The blocks produced were compact, not deliquescent, and hard enough to control their intake (to allow animals to lick, but not to cut/break and chew it). Blocks were turned from time to time to accelerate the drying process (Ben Salem et al. 2003).

2. 2. Description of animals

Twenty-four (24) lactating local zebu dairy cows reared under a semi-intensive production system were randomly selected from 17 farms (one to three animals were selected from one farm). The selected dairy cows had similar equal milk yields (3 ± 0.3 l/day), body condition score (BCS: 5 ± 0.5 out of 9) and age (4 ± 0.2 years). The cows were also reared under similar a condition which means that they grazed on the same communal range land and were supplemented with similar feed types and kept in self-constructed houses during night time.

2. 3. Feed management, and experimental design

This study was an intervention study designed to identify the effect of *M. stenopetela* MNB supplements on milk yield, body condition score, blood metabolite, and acylcarnitine profile of the lactating local zebu dairy cows. Four groups of six cows were each randomly assigned to one of the four MNB with different inclusion of *M. stenopetela* (M-0%, M-25%, M-35%, and M-45%). The *M. stenopetala* block was provided twice daily at 8 AM before animals were released to rangeland and at 6 PM when they turned back home, in an individual compartment. Blocks were offered for 2 h per day (1h morning and 1h afternoon) enabling the cows to adapt during the first 4 days, followed by 3 h per day (1:30 morning and 1:30h afternoon) to allow more adaption of blocks during the next 10 days. Thereafter, the cows were given a *M. stenopetala*

MNB after 6 PM at an individual compartment overnight (Ben Salem et al. 2003) and had free access to clean potable water throughout the experiment period (14 days). The first 14 days served as the diet adaptation period and the last 14 days served as the data collection period. The *M. stenopetala* MNB with about 2 kg (Faftine and Zanetti 2010) were fed to the animals and were replaced only when about one-quarter of the supplied quantity remained. Before the experiment, the animals were dewormed and vaccinated against common diseases of dairy cows in the area.

2. 4. Nutrient analysis of feed

Feed samples were analysed for dry matter (DM), ash, and ether extract (EE) according to (AOAC, 2005). The DM in feed samples was determined by oven-drying at 105°C overnight. Ash content was measured by incinerating samples at 550°C for 4 h. Nitrogen content in feed samples was determined using the Kjeldahl procedure using $CuSO_4$ as catalyst (AOAC 2005). The concentration of crude protein (CP) content in feed samples was calculated by multiplying the N concentration by 6.25 (Clark and Barbano, 1990). Neutral detergent fiber (NDF) content in feed samples was determined according to (Van Soest et al. 1991) (Table 2).

Table 2
Chemical composition of the diet components (% on DM basis) used in the study.

	Local feed mixture	MNB(M-0%)	MNB(M-25%)	MNB(M-35%)	MNB(M-45%)
DM	91.40	82.32	83.20	84.50	86.27
CP	6.37	32.10	34.35	37.13	38.76
NDF	75.45	19.23	18.26	17.44	17.98
EE	3.38	6.30	6.13	5.98	5.03
Ash	8.17	23.21	22.76	22.51	22.47

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; EE, ether extract; MNB(M-0%), Moringa multi-nutrient block without moringa leaf powder; MNB(M-25%), Moringa multi-nutrient block with 25% moringa leaf powder inclusion; MNB(M-35%), Moringa multi-nutrient block with 35% moringa leaf powder inclusion; MNB(M-45%), Moringa multi-nutrient block with 45% moringa leaf powder inclusion; MNB, multi-nutrient block.

2. 5. Blood sampling and acylcarnitine analysis

Blood samples were taken from the jugular vein of the animals before the start (day 1) and at the end of the experiment (day 28). The blood samples were collected in heparin tubes and placed in an icebox. The samples were centrifuged (1008 *g* for 10 min) immediately after the arrival at the laboratory. The plasma was collected and frozen at -20°C until analysis. Plasma concentrations of non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), urea, creatinine, triglyceride, and glucose were measured by spectrophotometry (UV-3100 PC, VWR).

Acylcarnitine profiles were conducted using dried plasma spots to get information about biomarkers as a relatively cheap and automated method (Dermauw et al. 2013). About 35 µL drops of serum were collected on Whatman™ protein saver TM903TM card for dried bloodspot analysis. Most of the analytes remain stable at room temperature for one week or more; in freezers, the analytes remain stable for long periods (McDade et al. 2007). Samples were subjected to tandem-mass spectrometry according to the method of Zytkovicz et al. (2001) at the Laboratory for Metabolic Diseases at Ghent University Hospital.

2. 6. Milk yield and body condition score

Dairy cows producing 3 ± 0.32L/day milk yields were selected, and daily milk yields were recorded during an experiment (14 days) and the average was calculated at the end of the experiment. Milking took place twice a day (morning at 7:00 am and afternoon 6:00 pm) in individual barns. The body condition of the animals was scored from 1 to 9 by 2 independent evaluators before supplementation (at day 1) and after supplementation (at day 28) for each individual cow based on the criteria set by Nicholson and Butterworth (1986), and in case of a deviation in score between 2 evaluators, they made a decision based on consensus.

2. 7. Data Analysis

Statistical analysis was performed using SPSS version 27. Two-way variance analysis was used to compare the effects of treatment and group on blood metabolites, acylcarnitine, body condition score, and milk yields of dairy cows. Post-hoc differences were evaluated using independent t-tests. Significance was accepted at the 5% probability level.

3. Results

The local feed mixture (a mixture of hay, grass from communal range land, crop residues and homemade by-product etcetera) was too low in CP content (6.37%) and too high in NDF content (75.45%) (Table 2). The milk yield was increased during the period of multi-nutrient block supplementation (Table 3). The significant time x treatment interaction was caused by a marked increase with the M-45% blocks compared to the M-35% blocks. Body condition score increased during multi-nutrient block supplementation, but independent of the multi-nutrient block type.

Table 3
Effect of supplementing multi-nutrient blocks on milk yield and body condition score of ranging dairy cows reared in the southern Ethiopian Rift Valley (n = 6).

	Before supplementing				After supplementing				SEM	*P*		
	M-0%	M-25%	M-35	M-45%	M-0%	M-25%	M-35	M-45%		T	*G*	T×G
Milk yield (l/d)	2.95	3.03	3.05	2.92	4.00	4.00	3.58	4.42	0.04	< 0.001	0.002	0.002
BCS	5.04	4.90	4.85	5.02	6.17	6.00	5.83	6.33	0.05	< 0.001	0.356	0.356

T, time (before and after supplementation); G, group (M-0%, M-25%, M-35% and M-45%); BCS, body condition score

During the supplementation period, cows got higher plasma glucose, triglyceride, and urea concentrations and lower concentrations of BHB, NEFA, and creatinine (Table 3), all independent of MNB type. During the MNB supplementation, isovaleryl carnitine + 2-methylbutyryl carnitine (C5), beta-hydroxybutyryl carnitine (3OHC4) and malonyl carnitine (C3DC) remained stable, whereas free carnitine (C0), acetyl carnitine (C2), propionyl carnitine (C3), methylmalonyl carnitine + succinyl carnitine (C4DC), glutaryl carnitine (C5DC) and methylglutaryl carnitine (C6DC) were increased at the end of the supplementation period. The concentration of glutaryl carnitine (C5DC) was increased in cows receiving M-0% and M-25% blocks and was significantly affected by treatment x group interaction (Table 4). By contrast, the concentration of butyryl carnitine + isobutyryl carnitine (C4) decreased during the supplementation period (Table 5).

Table 4

Effects of supplementing multi-nutrient blocks based on *Moringa stenopetala* on spectrophotometrically determined plasma metabolite concentrations in ranging dairy cows in the southern Ethiopian Rift Valley (n = 6)

Metabolite	Before supplementing				After supplementing				SEM	*P*		
	M-0%	M-25%	M-35%	M-45%	M-0%	M-25%	M-35%	M-45%		T	G	T×G
Glucose (mol/L)	2.69	3.07	2.82	3.01	3.96	4.08	3.66	3.53	0.057	< 0.001	0.166	0.148
BHB (mmol/L)	0.32	0.36	0.36	0.27	0.12	0.11	0.09	0.10	0.010	< 0.001	0.475	0.374
NEFA (mmol/L)	2.68	2.34	2.86	2.61	0.92	1.22	1.01	0.99	0.098	< 0.001	0.356	0.558
Triglycerides (mmol/L)	1.61	1.44	1.45	1.46	1.79	1.81	1.61	1.64	0.039	0.007	0.413	0.783
Creatinine (mg/dL)	2.85	2.35	2.25	2.50	2.17	1.93	1.91	1.90	0.064	< 0.001	0.097	0.788
Urea (mmol/L)	0.39	0.60	0.62	0.76	2.68	2.37	2.56	2.74	0.067	< 0.001	0.542	0.585

T, time (before and after supplementation); G, group (M-0%, M-25%, M-35% and M-45%); BHB, beta-hydroxybutyrate, NEFA; non-esterified fatty acids

Table 5

Effect of multi-nutrient block supplementation on plasma concentrations of selected carnitine esters in ranging dairy cows in the southern Ethiopian Rift Valley (n = 6)

Carnitine esters (µmol/l)	Before supplementing				After supplementing				SEM	*P*		
	M-0%	M-25%	M-35%	M-45%	M-0%	M-25%	M-35%	M-45%		T	G	T×G
C0	7.398	8.500	9.032	6.332	8.332	8.221	9.287	10.44	0.452	0.017	0.233	0.006
C2	0.522	0.451	0.57	0.44	0.77	0.84	0.82	0.72	0.332	0.015	0.092	0.080
C3	0.032	0.041	0.062	0.034	0.041	0.054	0.068	0.029	0.017	0.018	0.209	0.161
C4	0.552	0.559	0.501	0.510	0.551	0.489	0.458	0.379	0.014	0.049	0.057	0.450
C5	0.110	0.112	0.123	0.083	0.123	0.110	0.082	0.112	0.006	0.971	0.754	0.190
3OHC4	0.031	0.031	0.042	0.032	0.032	0.031	0.031	0.062	0.002	0.082	0.213	0.064
C3DC	0.012	0.013	0.011	0.021	0.011	0.021	0.011	0.012	0.001	0.122	0.670	0.432
C4DC	0.022	0.026	0.032	0.019	0.034	0.032	0.041	0.035	0.001	< 0.001	0.080	0.671
C5DC	0.003	0.002	0.010	0.005	0.015	0.010	0.010	0.007	0.001	0.002	0.023	0.046
C6DC	0.015	0.012	0.020	0.015	0.023	0.028	0.018	0.025	0.001	0.008	0.994	0.198

T, time(before and after supplementation); G, group(M-0%,M-25%,M-35% and M-45%);; C0, free carnitine; C2, acetyl carnitine; C3, propionyl carnitine; C4, butyrylcarnitine + isobutyryl carnitine; C5, isovaleryl carnitine + 2-methylbutyryl carnitine (isoleucine catabolism); C4DC, methylmalonyl carnitine + succinyl carnitine (valine and methionine catabolism); C5DC, glutaryl carnitine (lysine and tryptophan catabolism); C6DC, 3-methylglutaryl carnitine; 3OHC4, 3-hydroxybutyryl carnitine; C3DC, malonyl carnitine

4. Discussion

Measuring feed intake of free ranging cattle was not practically possible, because the animals were reared under uncontrolled environmental conditions and were grazing with other livestock on communal range lands. The cows were also reared on local feeds (local feed mixture; grass from communal grazing land and supplemented with hay, crop residue and home leftover when back to home at night), and the availability of these feed resources were dependent on the season of the year (Ketema et al. 2021). Due to these circumstances, measuring feed intake was not possible but the concept of this study was to see how providing *M. stenopetala* leaves based MNB could affect performance and metabolism under practical conditions.

Since the local feed was too low in protein to support proper rumen microbial function and too high in fibre to provide enough digestibility (Osuji et al. 1995; FAO 2014), the use of high protein content and lower fibre content in the MNB were meant to adjust these shortcomings. During the period of MNB supplementation, the dairy cows indeed improved their condition and performance. We are aware that no non-supplemented group was included as a negative control, but given the magnitude and consistency of the effects, we still dare to conclude that the use of the MNB has had a marked beneficial effect for the ranging dairy cows. Evidently, we cannot exclude potential side-effects generated by the participating farmers, since this was obviously not a blinded study, so farmers may have done more efforts to care for their livestock while participating in this study (Glover 2015). However, whether the improvement is directly or indirectly caused by the MNB use, the impact is important and supports the use of (any) balanced MNB. The improved body condition and milk yield are reflected in the metabolite profiles: elevated glucose and triglyceride concentrations are needed for the synthesis of lactose and milk fat respectively (Jaakson et al. 2013).

The MNB supplementation appears to allow higher milk yield without being at the expense of body reserves since typical mobilisation markers such as NEFA, BHB and creatinine (Adewuyi 2005; Reist et al. 2002; Agenas et al. 2006) are even lower than at the start. The increased urea thus points to the higher availability of amino acids from dietary protein as an energy source (Hammond, 2006; Chonyo et al. 2002). Although dietary protein is preferable to be used for milk protein synthesis, it is a crucial factor in maintaining the citric acid cycle in the absence of a good source of gluconeogenesis (Chandler and White 2019). In intensive dairy husbandry, the use of fast-fermentable resources stimulates propionate production in the rumen to act gluconeogenic (Van Knegsel et al. 2007; Bannink, 2006).

Acylcarnitine profiling has been employed in several animal species to study nutrient metabolism, ranging from cats (Verbrugghe et al. 2009) to fish (Geda et al. 2017). In the present study, the concentration of propionyl carnitine (C3) – as a marker of propionate production from fermentation (Verbrugghe et al. 2012) was increased during the supplementation period, indeed suggesting a higher provision of this gluconeogenic substrate, but maybe not enough to meet the demands of the citric acid cycle. This hypothesis is supported by the increased concentrations of glutaryl carnitine (C5DC), an intermediate of lysine and tryptophan catabolism (Millington and Stevens 2011). The elevated concentration of methylmalonyl + succinyl carnitine shows an increased inflow of propionate, valine, or methionine as gluconeogenic substrates for the citric acid cycle (Waters et al. 2016). The increased concentration of methylglutaryl carnitine (C6DC) suggests that even an excess of leucine could be used as a lipogenic energy source, leading to a higher supply of acetyl coenzyme A, as shown by the increase in acetyl carnitine (C2). We anticipated that malonyl carnitine (C3DC) would, therefore, have increased in association with the higher milk production, higher triglyceride concentrations, and lipogenic substrates, but that component remained unchanged. Malonyl coenzyme A is proposed as a marker for lipogenesis (Bandyopadhyay et al. 2006; Malewiak et al. 1985), but maybe in our study, this has not been a limiting step in lipogenesis. In connection to that, it is remarkable that BHB dropped during the supplementation period, whereas no significant change was observed in the corresponding carnitine ester, 3-hydroxy-butyryl carnitine (3OHC4). A possible explanation for this difference is the fact that BHB may act independently of the carnitine shuttle (Agosti et al. 1966).

Although free carnitine (C0) increased with all MNB types, this increase was most prominent in the highest inclusion of *M. stenopetala* leaves, which was also associated with the highest increase in milk yield. Since herbivore diets contain negligible amounts of carnitine (Lombard et al. 1989; Steiber et al. 2004), an increase in free carnitine can originate from a decreased degree of esterification or a higher *de novo* synthesis. Since the total sum of carnitine esters was higher, the option of decreased esterification is unlikely, especially given the higher metabolic rate associated with increased lactation. *De novo* synthesis of carnitine depends mainly on two amino acids, lysine, and methionine. It was shown that the amino acid pattern of *M. stenopetala* leaves is well-balanced and even comparable to the soybean amino acid profile (Abere et al. 2012). Therefore, the highest inclusion of *M. stenopetala* leaves may have stimulated carnitine biosynthesis. It is yet unclear if this has been instrumental in any way to the higher milk yield observed in the highest *Moringa* inclusion group.

Conclusions

The four examined MNB showed significant variances in the overall image of this study, and it was quite obvious that *M. stenopetala* leaves were included at the highest rate. The fact that the highest inclusion of leaves of this fodder tree in the tested MNB gave the best performance and associated metabolite profile, on top of the already improved performance during the MNB use, shows the promising potential of this local resource as a basis for MNB to increase the performance of tropical ranging dairy cattle, hence as an indirect strategy to reduce overgrazing.

Declarations

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Author contribution

Ketema Worku conceptualized the idea of the study, curated the data, performed formal analysis and investigation, designed methodology, administered the project, and wrote the original draft. **Yisehak Kechero** and **Geert PJ Janssens** acquired funding, performed writing, paper drafting, and reviewed and edited the manuscript. Each author made a significant contribution to the creation of the data, the writing of the paper, and its final revision. Each author also gave their approval for the submission of the work for journal publication.

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Data availability

Upon reasonable request, the corresponding author will provide the data that back up the study's findings.

Ethics approval

The authors affirm that the journal's ethical rules, as stated on the author guidelines page, were followed. The authors affirm that the journal's ethical rules, as stated on the author guidelines page, were followed. The animal study protocol was approved by the Institutional Review Board of College of agricultural Sciences of Arba Minch University, AMU-CoA (protocol code 13128 on 08–02-2022). Consent to participate is not applicable.

Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability

All of the data created or analyzed throughout this investigation are presented in this publication and are further available from the relevant author upon request.

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