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ORIGINAL RESEARCH

Can cattle blood be transformed into an organic source of nitrogen?

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Abstract

Purpose: A study was conducted to transform bovine blood, a slaughterhouse by-product, into an organic source of nitrogen, which is otherwise disposed of through sewer systems only to pollute the nearby water bodies.

Method: The annual blood production in the Khulna City Corporation (KCC) slaughterhouses was estimated, blood samples were collected, and blood meals were manufactured and characterized for their nitrogen content and other nutritional values. The best blood meal was sorted out, incubated in soil, and applied to spinach (*Spinacea oleracea* L.) to assess the effects of the final product on plant and soil health.

Result: This investigation estimated the annual disposal of 58.62 tons of bovine blood from the KCC slaughterhouses. The conventionally derived blood meal (BMc) attained the higher amount of primary nutrients (NPKS), while oven-dried blood meal (BMod) attained the higher amount of secondary nutrients (Ca and Mg), micronutrients (Fe, Cu, and Mn) and heavy metals (Cr, Pb, Ni). An increasing rate of blood meal incubation in soil increased available N and N-mineralization with an incubation time of up to 90 days. Blood meal application to spinach at a rate of 5 t/ha had evident higher productivity and better N-utilization efficiency although application rate above 5 t/ha declined crop performance.

Conclusion: The outcome of the study suggests that blood meal can be used as an organic source of nitrogen and the application of blood meal has manifold benefits if applied at a judicious rate preferably less than or equal to 5 t/ha.

Keywords: Waste recycling, Sustainable agriculture, Healthy soil, Environmental benefits

Introduction

As blood meal is an inexpensive source of nitrogen for plants, the proper management of bovine blood is obvious rather than its scientific discharge, which causes rigorous environmental pollution and serious public health hazards (Bhunia et al. 2022). Like in other developing countries, cattle blood is produced and wasted in the slaughterhouses of Bangladesh. The total weight of blood from domestic animals is equivalent to 6 to 7% of the lean meat in the carcass (Bari et al. 2015). The majority of the research work focused on protein isolation from blood waste for fish meal production (Bari et al. 2015; Aladetohun and Sogbesan 2013), while our study intended for organic fertilizer development for sustainable livelihood in the climate change milieu.

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Blood meal can be manufactured by drying and powdering the blood of slaughtered animals and can be used as organic nitrogen (N) fertilizer as it contains about 10-13% organic N, which normally release during the mineralization of organic matter (Ciavatta et al. 1997). In Bangladesh, the conversion of cattle blood into blood meal, i.e., its transformation from a water contamination agent to a crop vitalizer, was not attempted as of today.

Chemical fertilizers sustain the short-term productivity of agroecosystems, while their indiscriminate use reduces soil fertility, adversely affects enzymatic activity, and jeopardizes copiotrophic communities (Ansari and Mahmood 2017). Synthetic fertilizers can pollute the surface soil as well as groundwater (Tal 2018). Some fertilizers contain heavy metals (cadmium and chromium) and high concentrations of radionuclides (Savci 2012). The majority of these inorganic substances are persistent (Geiger et al. 2010), and not readily degraded by natural microorganisms, which can reduce soil viability and negatively affect the quality of produce (Kim et al. 2014).

A goal of concurrent agriculture is to meet society's present and future food demands with a surplus amount of availability for exporting. Sustainable agriculture requires the careful optimization of the use of organic amendments to improve soil fertility while minimizing any harmful environmental effects (Masungaa et al. 2016). The use of blood meal in agronomy can partially reduce the dependence on inorganic fertilizers. In addition, zero waste management is a distinctive philosophical concept that varies in different aspects. Generally, zero waste management is the technique by which all the discarded materials are used for productive purposes through material conversion or direct use of them (Giampietro and Ulgiati 2005). Although information regarding the chemical structure and composition of blood meal is available from biochemical investigations (Jameel 2019; Mishra et al. 2015), knowledge about the methods of blood meal preparation, dose calculation, and application frequency determination, and effect analysis on plant and soil health are fairly limited (Ciavatta et al. 1997).

Khulna is the third largest metropolitan city in Bangladesh and Khulna City Corporation (KCC) is the responsible authority to manage the city waste. Khulna City slaughterhouse waste has been classified into three categories—blood volume (19.55%), intestine content (50.81%), and ruminal content (29.64%) (Amin 2009). The solid part is collected and processed by the KCC authority while the liquid part, blood, has so far been allowed to run down and pollute nearby water bodies. No record of the annual production of waste was found. Lack of proper waste management and no resource recovery initiatives in operation have been noticed in the KCC slaughterhouses.

Therefore, this study aimed to meet the following specific objectives:

a) To estimate the annual cattle blood production in KCC slaughterhouses.
b) To evaluate the potential of the methods applied for blood meal production and characterize them.
c) To incubate different rates of blood meals for the assessment of the effects of blood meal doses on N- availability, and mineralization.
d) To find out the effects of different rates of blood meals on crop growth performances and nitrogen relationship indicators.

Materials and Methods

Collection of cattle blood and slaughterhouse survey

There are three slaughterhouses in KCC located in the areas of Gollamari, Khalispur, and Rupsa (Fig. 1). Among them, Rupsa was inoperative during the study period.

However, before conducting the survey, information about the number of cattle slaughtered in the year 2019-2020 was acquired from the Food Safety Department, KCC. The number of cattle slaughtered in a day, their live weight, and the amount of blood collected after each slaughter were recorded for randomly selected 15 days. The cattle were then categorized by their live weight. The amount of blood that can be collected from the KCC per annum was then estimated. Fresh liquid blood of cattle (cow, goat, and buffalo) was collected from the Gollamari slaughterhouse near Khulna University. The cattle were slaughtered on a thick poly sheet connected to a collection polybag, and blood was collected with minimum contamination of other waste materials. The collected blood samples were processed on the same day for blood meal production. To avoid putrefaction, collected blood was treated with lime (0.5%) and stored in a closed (air-tight) container.

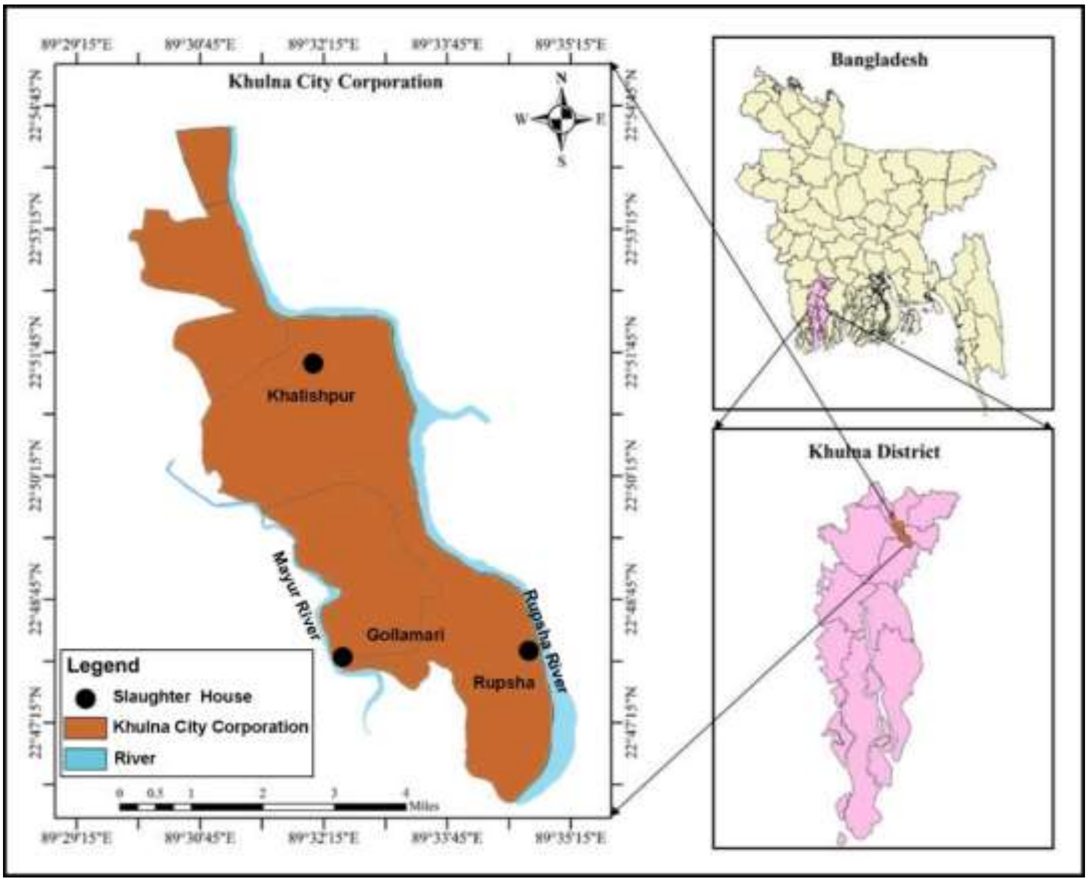

Fig. 1 Map of Khulna City Corporation showing the location n of the slaughterhouses

Blood meal preparation

Three different methods were carried out to produce blood meals with different temperatures: the conventional method (>100 ºC), the water bath method (100 ºC), and the oven drying method (65 ºC). In the conventional method (BMc), fresh liquid blood pretreated with lime was taken into an aluminium saucepan and heated on a gas stove. The liquid blood was stirred continuously until excess moisture was removed. It took about 30-40 minutes to remove the excess moisture (IGNOU 2017). In the water bath method (BMwb), fresh blood was taken into a beaker and heated for 4-5 hours on a water bath set at 100 ºC to remove excess moisture. In the oven-dry method (BMod), pretreated fresh blood was taken into a beaker and kept in an electric oven for 24 hours at 65 ºC to remove the excess moisture. In all cases, the semi-dried blood slurry was taken in separate steel trays and sundried for 3 days to produce blood meal.

Evaluation of the applied methods

The methods employed for the production of blood meal were evaluated as on their yield potential and upon characteristics of the blood meal produced. Index values were estimated and used for the evaluation of the methods as well as blood meal for incubation study and pot application. In doing this, the content of each nutrient (N*i*) was multiplied by the percent blood meal yield of each method (Y*i*). Similarly, index values for heavy metals were also estimated. For the estimation of annual blood production, the average amount (kg) of collectible blood from each type of cattle was estimated and multiplied by the total number of cattle slaughtered. The amount of blood meal produced from fresh cattle blood was calculated by the following equation (1) (IGNOU 2017):

$$Yield\ of\ Blood\ meal\ (\%) = \frac{Dry\ weight\ of\ blood\ meal\ (kg)}{Initial\ weight\ of\ liquid\ blood\ (kg)} \times 100 \quad (1)$$

The annual blood meal production was estimated by multiplying the yield with the annual blood production.

Characterization of blood meal

The blood meals produced by conventional cooking (BMc), water bath (BMwb), and oven-drying (BMod) methods were pulverized and sieved through a 0.5 mm sieve. The blood meals were then analyzed for soil reaction (pH), electrical conductivity (EC), organic carbon (OC), primary (N, P, K) and secondary (Ca, Mg, S) macronutrients, micronutrients (Fe, Cu, Zn, Mn) and heavy metals (Cr, Pb, Cd, Ni).

Application of blood meal as fertilizer

Soil sampling and characterization

Surface soil (0-15 cm) was collected from a medium high land, poorly drained, crop field (22º47'44"N and 89º27'35"E.) of Jilerdanga village of Dumuria Upazila, Khulna, Bangladesh. The physiography, parent material, agro-ecological zone, and soil series of the sampling location are the Ganges Tidal Floodplain, Ganges Tidal Alluvium, AEZ-13, and Bajoa series (SRDI 2008), respectively. The massive aggregates were broken by gentle crushing with a wooden hammer, and non-soil materials were removed by sieving through a 2.0 mm sieve and then air-dried. The processed soil samples were preserved for i) initial characterization, ii) an incubation study, and iii) a pot experiment. A small portion of it was taken for routine analysis (soil texture, pH, EC, OC) as well as for major nutrient (N, P, K, S) analysis by the following standard methods. The results are presented in Table 1.

Table 1 Soil properties

Texture	pH	EC (dS/m)	OC (%)	Total N (%)	Available P (ppm)	Available K (ppm)	Available S (ppm)
Clay loam	7.53	1.74	1.44	0.10	19.4	201.81	231.06

Incubation study

Among the three different blood meals, only the cheap and easy blood meal (BMc) was used for both the incubation study and the pot experiment. The blood meal was thoroughly mixed with the soil at different rates and incubated for different periods (15-, 30-, 45-, 60-, 90-, and 120-days). Following a completely randomized design (CRD) method, a total of 24 pots (6 treatments × 4 replications) were filled with 2 kg processed soil (Table 2). Incubated soil samples at different intervals were collected and characterized. The effects of blood meal application on soil pH, EC, OC, available N, and % N mineralization were assessed.

The percentage of N mineralization at different incubation times ($N_{min}t$) was calculated as described by Lazicki et al. (2020) (Equation 2).

$$\text{N}mint\ (\%) = \frac{AN(amended) - AN(control)}{N\ Total\ (applied)} \times 100 \quad (2)$$

where, **N*mint*** is nitrogen mineralization; ***AN (amended)*** is available nitrogen (NH_4-N+NO_3-N) in soil amended with blood meal; ***AN (control)*** is available nitrogen (NH_4-N+ NO_3-N) in control soil; and '***N Total***' is total nitrogen in the applied blood meal for respective treatment.

Pot experiment

During the pot trial, Spinach (*Spinacia oleracea* L. var Khupipalong) locally known as Palong was selected as a test crop, one of the most widely consumed vegetables in Bangladesh. Following a completely randomized design (CRD) method, a total of 30 pots (10 treatments × 3 replications) were filled with 6 kg of processed soil (Table 3). P and K were supplied at the recommended rate (BARI 2018) to all treatments using triple superphosphate (TSP) and muriate of potash (MOP) as sources. Two sets of these treatments were created: one to compare the blood meal treatments, and the other to compare chemical and organic fertilizers. The treatments – cow dung, poultry manure, and vermicompost used in this experiment, respectively contains 1.33%, 2.59%, and 1.56% nitrogen. Inorganic fertilizers were applied to soil following the guidelines of BARI (2018).

Table 2 Blood meal treatments applied to soil for incubation

Treatment No.	Treatment Notation	Blood meal treatments
1	T_C	Soil (Control)
2	T_{BM1}	Soil + Blood meal (1 t/ha)
3	T_{BM2}	Soil + Blood meal (5 t/ha)
4	T_{BM3}	Soil + Blood meal (10 t/ha)
5	T_{BM4}	Soil + Blood meal (15 t/ha)
6	T_{BM5}	Soil + Blood meal (20 t/ha)

Table 3 Treatments used in the experiment

Treatment No.	Treatment Notation	Treatments
1	T_C	Control (Soil)
2	T_{BM1}	Soil + Blood meal (1 t/ha)
3	T_{BM2}	Soil + Blood meal (5 t/ha)
4	T_{BM3}	Soil + Blood meal (10 t/ha)
5	T_{BM4}	Soil + Blood meal (15 t/ha)
6	T_{BM5}	Soil + Blood meal (20 t/ha)
7	T_{CD}	Soil + Cow Dung (10 t/ha)
8	T_{VC}	Soil + Vermicompost (10 t/ha)
9	T_{PM}	Soil + Poultry Manure (10 t/ha)
10	T_U	Soil + Urea (180 kg/ha)

Seven seeds of the test plant were sown in each pot. After germination, seedlings were thinned, ensuring an equal number of seedlings (5) in each pot. Weeds were removed manually.

Irrigations were applied when required throughout the growing period. The plants were harvested after 49 days (7 weeks) of seed sowing. Harvested plant samples were separated for growth parameter assessment and laboratory analysis. The agronomic growth parameters—leaves per plant, plant height (before harvesting) and root length, fresh yield, dry matter content (Equation 3), and dry matter yield (after harvesting) were estimated timely.

$$Dry\ matter\ content\ (\%) = \frac{Dry\ Weight\ (g)}{Fresh\ Weight\ (g)} \times 100 \quad (3)$$

Nitrogen uptake and utilization efficacy assessment

Nitrogen (N) uptake and N utilization efficiency (NUtE) were calculated by equation (4) (Solangi et al. 2015) and equation (5) (Abdelraouf 2016), respectively. The nitrogen transfer factor and translocation factor were calculated following equation (6) and equation (7), respectively (Mirecki et al. 2015).

$$N\ uptake (kg/ha) = \frac{Dry\ matter\ yield\ (kg/ha) \times N\ concentration\ (\%)\ in\ plant}{100} \quad (4)$$

$$NUtE\ (kg/kg) = \frac{Dry\ matter\ yield\ (kg/ha)}{N\ uptake\ (kg/ha)} \quad (5)$$

$$Transfer\ Factor = \frac{N\ concentration\ in\ plant}{N\ concentration\ in\ Soil} \quad (6)$$

$$Translocation\ Factor = \frac{N\ concentration\ in\ shoot}{N\ concentration\ in\ root} \quad (7)$$

Laboratory analysis

The particle size analysis was carried out by hydrometer method as described by Gee and Bauder (1986). The textural class was determined using Marshall's Triangular Coordinator system. The pH of the soil and blood meal were determined by a glass-electrode pH meter maintaining a soil-water ratio of 1:2.5 (McLean 1982).

The electrical conductivity of the soil and blood meal were measured at a soil-water ratio of 1:5 and blood meal-water ratio of 1:5 respectively by EC meter (USDA 2004).

The organic carbon content of soil and organic matter content of blood meal were determined by using Walkley and Black wet oxidation method (Walkley and Black 1934).

The total nitrogen content in the soil, blood meal and plant sample was determined by the Micro-Kjeldahl method (Bremner and Mulvaney 1982). Available phosphorus was extracted from the soil with 0.5 $NaHCO_3$ (Olsen extractant) at pH 8.5 and determined by the ascorbic acid blue color method (Olsen et al. 1954).

Blood meal was digested with nitric-perchloric acid (2:1) as described by Piper (1966). Total phosphorus in the blood meal was determined by the Vanadomolybdophophoric Yellow Color method and total sulfur of blood meal was determined by the turbidimetric method as described in Jackson (1973).

The available sulfur of the soil sample was determined by the turbidimetric method as described by Page et al. (1982).

The available K^+ of the soil sample was determined from NH_4OAc (pH 7.0) extract as described by Jackson (1973). From the digest total K, Ca, Mg, Fe, Cu, Mn, Zn, Cr, Pb, Cd and Ni content were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Aydin et al. 2010).

Statistical analysis

The collected data of different parameters were statistically analyzed for one-way and two-way ANOVA for respective purposes. The difference among the treatment means was compared by using Duncan's Multiple Range Test (DMRT) at a probability level of 0.05 (Gomez and Gomez 1984). The data were statistically analyzed and presented by using different statistical softwares such as SPSS 16.0, Statistix 10.0 and MS Excel.

Results and Discussion

Survey result

Each of the KCC slaughterhouses was found to be devoid of any management system for the liquid waste, which in turn contaminates nearby river water (Fig. 2) and the environment around it. The solid waste from the slaughterhouses is collected by KCC, and disposed of and processed at the waste dumping site.

The survey study revealed that cow constitutes 89.48% of total blood produced per anum (Table 4) and estimation from the survey data projects that KCC slaughterhouses annually produces 58.6 tons of conventionally collectible fresh cattle blood, which upon conversion can produce 10.7 tons of bloodmeal (Table 4). This finding is in close agreement with the result of blood waste production (65.2 tons) found by Amin (2009). He also stated that the pungent odor, severe water pollution, drainage congestion, and eutrophication as a consequence caused by the KCC slaughterhouse wastes, were the prominent features and the main problems of the Mayur River at Gallamari site (Amin 2009).

Fig. 2 Water Pollution in the Mayur River caused by blood spillage from Gollamari slaughterhouse

Table 4 Amount of cattle blood produced in KCC in 2019-2020

Cattle type	No. of cattle slaughtered/year	Blood produced (kg/year)	Blood produced (%)	Blood meal (kg/year)
Cow	13986	52447.50	89.48	9555.93
Goat	17659	6004.06	10.24	1093.94
Buffalo	44	165.00	0.28	30.06
Total	**31689**	**58616.56**	**100**	**10679.94**

Evaluation of the methods applied for blood meal production

According to the results obtained for blood meal yield (%),the ‘oven drying’ method produced significantly ($p<0.05$) higher blood meal (23.11% ±0.69) than the ‘conventional’ and ‘water bath’ method (Table 5). The pH of blood meal ranged from 7.44 to 7.75, making itslightly alkaline. The electrical conductivity (EC) ranged between 6.63 and 6.8 dS/m equivalent to 0.42% and 0.44% salt respectively, which indicated the presence of high salt content in the blood meal. Organic matter content ranged from 34.29% to 38.63%.

The BMc had significantly (p<0.05) higher pH, EC, N, P, K, and S contents and significantly lower organic matter than that of the water bath method. The BMod contained significantly (p<0.05) higher Ca, Mg, micronutrients (Fe, Cu, and Mn) and heavy metals (Cr, Pb, Cd and Ni) (Table 5).

Almost similar results were obtained for total N (14.9%; 12.93%) (Ciavatta et al. 1997; Citak and Sonmez 2010), organic matter (41.0%), pH (6.50), and EC (6.0 dS/m) (Citak and Sonmez 2010). The concentration of secondary (Ca, Mg) and micronutrients widely varied from those obtained by Ciavatta et al. (1997).

The highest index values for both essential nutrients and heavy metals were obtained with BMod followed by BMc and BMwb (Table 5). Despite having an intermediate index value, BMc was selected for incubation study and crop response study because of its much lower heavy metal content, cost-effectiveness and non-technical method of preparation.

Table 5 Properties of blood meals and indexing of methods

Properties	**Methods of blood meal production**					
	Conventional (>100 °C)		**Water bath (100 °C)**		**Oven drying (65 °C)**	
% Yield (*Y* i)	18.22±1.35 b		18.78±1.26 b		23.11±0.69 a	
pH (1:2.5 water)	7.75±0.06 a		7.63±0.04 b		7.44±0.04 c	
EC (1:5 water) (dS/m)	6.8±0.04 a		6.63±0.02 b		6.69±0.04 b	
OM (%)	36.30±1.08 b		38.63±0.81 a		34.29±1.02 c	
Indexing						
Properties	**Contents (*Ni*)**	**Index value (*Ni* x *Yi*)**	**Contents (*Ni*)**	**Index value (*Ni* x *Yi*)**	**Contents (*Ni*)**	**Index value (*Ni* x *Yi*)**
Primary nutrients (Total)						
N (%)	13.98±0.27 a	254.72	11.61±0.24 c	218.04	12.50±0.42 b	235.00
P (%)	0.104±0.007 a	1.895	0.091±0.008 a	1.709	0.074±0.005 b	1.391
K (%)	0.015±0.001 a	0.273	0.011±0.000 c	0.207	0.013±0.001 b	0.244
S (%)	0.182±0.002 a	3.316	0.089±0.000 c	1.671	0.131±0.003 b	2.463
Secondary nutrients (Total)						
Ca (ppm)	1388.80±56.38 b	25303.94	1397.50±67.37b	26245.05	1687.50±70.36 a	31725.00
Mg (ppm)	361.51±14.21 b	6586.71	313.65±13.42c	5890.35	485.92±17.40 a	9135.30
Micronutrients (Total)						
Fe (ppm)	2296.20±78.81 b	41836.76	2291.20±33.75 b	43028.74	2443.00±86.47 a	45928.40
Cu (ppm)	149.59±7.23 c	2725.53	165.37±5.05 b	3105.65	254.19±5.06 a	4778.77
Zn (ppm)	1309.10±51.31 a	23851.80	564.27±13.49 c	10596.99	666.53±11.05 b	12530.76
Mn (ppm)	6.19±0.09 b	112.78	5.87±0.12 c	110.24	9.37±0.20 a	176.16
Total index value		**100677.73**		**89198.63**		**104513.49**
Heavy metals/trace elements (Total)						
Cr (ppm)	7.38±0.29 b	134.46	7.50±0.23 b	140.85	8.82±0.17 a	165.82
Pb (ppm)	57.97±3.50 b	1056.21	60.04±1.54 b	1127.55	89.07±1.73 a	1674.52
Cd (ppm)	41.77±1.68 a	761.05	43.27±1.45 a	812.61	43.12±0.93 a	810.66
Ni (ppm)	9.44±0.47 c	172.00	11.34±0.42 b	212.97	14.48±0.45 a	272.22
Total index value		**2123.72**		**2293.98**		**2923.21**

Similar letters after the values in a row are not significantly different at p<0.05 according to Duncan's multiple range test

Evaluation of blood meal for soil benefits

Soil pH and electrical conductivity (EC)

The soil pH values obtained at different incubation periods were found to be higher than the initial value (0- day) of the experimental soil and the pH class was '*slightly alkaline*' according to USDA (1998) classification system. With the application of blood meal treatments (T_{BM1}, T_{BM2}, T_{BM3}, T_{BM4}, and T_{BM5}), pH values gradually declined throughout the incubation time and shifted to neutral class (Table 6) at the end of the incubation period.

A reverse trend was observed for soil EC values - a gradual increase with increasing incubation period was noticed from 15 days to 120 days of incubation. Thus, the EC values obtained for all treatments at 120 days were significantly higher ($p<0.0001$) than the values obtained at 15 days incubation period (Table 6). It was observed that application of blood meal at a rate equal to 5 t/ha (T_{BM2}) converted the *non-saline* soil into *very slightly saline* (2-4 dS/m) and when applied at a rate > 5 t/ha (T_{BM3}, T_{BM4}, and T_{BM5}) soil salinity moved into *slightly saline* class according to USDA (1998). Azeez and Van Averbeke (2012) found that soil EC significantly increases with the application of poultry, cattle and goat manures and the potential of manure-induced soil salinization was very high with poultry manure and goat manure compared with cattle manure. Dikinya and Mufwanzala (2010) revealed increased electrical conductivity with increasing rates of chicken manures. Moreover, a higher rate of blood meal application is anticipated to escalate soil salinity in coastal belt soils limiting suitability to most cereal crops and vegetables (Smith and Doran 1996).

Table 6 Changes in soil pH and electrical conductivity (EC) at different times of incubation

Blood meal treatments	Time of incubation					
	15 days	30 days	45 days	60 days	90 days	120 days
Soil pH						
T_C (0 t/ha)	7.85 ± 0.06 a	7.80 ± 0.08 a	7.68 ± 0.05 b	7.65 ± 0.06 bc	7.58 ± 0.05 cd	7.55 ± 0.06 d
T_{BM1} (1 t/ha)	7.58 ± 0.05 a	7.53 ± 0.05 ab	7.43 ± 0.05 bc	7.40 ± 0.08 c	7.33 ± 0.10 cd	7.28 ± 0.10 d
T_{BM2} (5 t/ha)	7.33 ± 0.05 a	7.28 ± 0.05 ab	7.25 ± 0.06 abc	7.23 ± 0.05 bc	7.20 ± 0.08 bc	7.18 ± 0.05 c
T_{BM3} (10 t/ha)	7.08 ± 0.05 a	7.05 ± 0.06 ab	7.03 ± 0.05 abc	6.98 ± 0.05 bcd	6.95 ± 0.06 cd	6.93 ± 0.05d
T_{BM4} (15 t/ha)	7.03 ± 0.10 a	6.95 ± 0.06 ab	6.93 ± 0.05 ab	6.85 ± 0.06 bc	6.80 ± 0.08 cd	6.73 ± 0.10 d
T_{BM5} (20 t/ha)	6.98 ± 0.05 a	6.95 ± 0.06 a	6.80 ± 0.08 b	6.73 ± 0.10 bc	6.65 ± 0.06 cd	6.60 ± 0.08 d
EC (dS/m)						
T_C (0 t/ha)	1.75± 0.01 d	1.77± 0.01 cd	1.79± 0.03 bc	1.79± 0.02 bc	1.81± 0.02 ab	1.83± 0.02 a
T_{BM1} (1 t/ha)	1.77± 0.01 d	1.86± 0.04 c	1.91± 0.01 b	1.94± 0.02 ab	1.97± 0.03 a	1.98± 0.04 a
T_{BM2} (5 t/ha)	1.87± 0.09 d	2.00± 0.12 c	2.07± 0.10 c	2.11± 0.07 c	2.42± 0.06 b	2.47± 0.03 a
T_{BM3} (10 t/ha)	1.89± 0.07 c	2.90± 0.14 b	3.38± 0.85 b	4.07± 0.18 a	4.31± 0.15 a	4.38± 0.07 a
T_{BM4} (15 t/ha)	2.14± 0.05 d	4.13± 0.23 c	4.30± 0.27 bc	4.53± 0.12 b	4.90± 0.16 a	4.94± 0.11 a
T_{BM5} (20 t/ha)	2.39± 0.06 d	5.23± 0.32 c	5.35± 0.25 bc	5.56± 0.17 ab	5.75± 0.06 a	5.80± 0.03 a

Similar letters after the values in a row are not significantly different at $p<0.05$ according to Duncan's multiple range test

The two-way ANOVA study showed that blood meal treatments and incubation time individually affected soil pH and EC while their interactions (treatment x incubation time) significantly ($p<.0001$) affected only EC (Table 7). Roy and Kashem (2014) reported similar findings. In their research, they observed that regardless of the type of manure, soil pH improved somewhat with the incubation duration up to 30 days before significantly declining over time ($p < 0.05$), but soil EC increased significantly (p 0.05) with increasing days of incubation.

Table 7 Two-way ANOVA of effects of blood meal treatments, time of incubation, and their interactions on soil pH, EC, SOC, available N and N mineralization rate

	Source of variation					
Soil properties	**Blood meal treatments (A)**		**Time of incubation (B)**		**A×B**	
	F	**p**	**F**	**p**	**F**	**p**
pH	647.88^{***}	0.0000	55.57^{***}	0.0000	1.55^{ns}	0.0664
EC	1250.76^{***}	0.0000	243.79^{***}	0.0000	39.24^{***}	0.0000
SOC	218.83^{***}	0.0000	292.72^{***}	0.0000	2.58^{***}	0.0004
Available N	23695.39^{***}	0.0000	10808.17^{***}	0.0000	251.23^{***}	0.0000
% N mineralization	17.86^{***}	0.0000	2078.32^{***}	0.0000	13.32^{***}	0.0000

Note: Data represent F-values at 0.05 level.

$^{***}p<0.001$; ns- not significant.

Soil organic carbon (SOC) content, available N and % N mineralization

Irrespective of the blood meal treatments, the higher SOC contents (%) were found in the soils of 15-day incubation and then continually declined with increasing incubation time, and thus the lowest SOC was measured at a 120-day incubation period (Table 8).

The higher amount of SOC at the beginning of the incubation was indicative of a larger pool of the less resistant fractions that were available to be broken down and recycled resulting in lower OC contents remaining at the end of incubation. Even the highest dose of blood meal failed to maintain SOC level (%) with progressing incubation time. It might be due to the fact that the addition of higher OC triggered the microbial activity which transformed the OC into microbial biomass carbon. The two-way ANOVA study showed that blood meal treatments, incubation time, and their interactions (treatment × incubation time) significantly ($p<0.0001$) affected SOC, available N and the % N mineralization (Table 7). Similar results were observed in several studies (Follett et al. 2007; Gulser et al. 2010; Manivannan et al. 2009), where they claimed that the effects of manure application on SOC significantly varied with manure type and that the addition of organic residues increases the SOC level initially and with time OC content decreases in soil up to a certain period. Roy and Kashem (2014) in their study found that OC contents of manure-treated soils reached their peak at 15 days of incubation and decreased thereafter with time. This study showed that the increase in available N contents in response to T_C (control) with increasing incubation time was significantly lower and different ($p<0.05$) than those with blood meal treatments (T_{BM1}, T_{BM2}, T_{BM3}, T_{BM4}, and T_{BM5}). The concentration of available N (mg/kg) increased gradually with increasing incubation time under all blood meal treatments (Table 8) with the fact that the higher the blood meal treatments (t/ha) the higher the availability. Available N contents significantly varied ($p<0.05$) when values (mg/kg) against increasing treatments were compared for all individual incubation steps. Available N followed an inverse and a proportional relationship between SOC (%) and EC respectively. Eigenberg et al. (2002) suggested monitoring soil N level by using EC measurements as they found a sound positive relationship between N-content with EC. Roy and Kashem (2014) found that NH_4^+-N increased significantly ($p < 0.05$) as the incubation period increased in control and cow dung amended soils and found a higher amount of NH_4^+-N after 60 days of incubation, with cow dung plus chicken manure treated soil followed by chicken manure treatment. Duffera et al. (1999) also reported the increased concentrations of NH_4^+-N after the first 15 days and the concentrations dropped by the next 15 days after application of manures.

Table 8 Changes in soil organic carbon (SOC) content, available N and % N mineralization at different times of incubation

Blood meal treatments	Time of incubation					
	15 days	30 days	45 days	60 days	90 days	120 days
SOC (%)						
T_C (0 t/ha)	1.49± 0.02 a	1.45± 0.01 b	1.39± 0.01 c	1.36± 0.02 d	1.27± 0.01 e	1.26± 0.02 e
T_{BM1} (1 t/ha)	1.44± 0.01 a	1.42± 0.01 a	1.34± 0.08 b	1.29± 0.01 b	1.22± 0.02 c	1.20± 0.02 c
T_{BM2} (5 t/ha)	1.38± 0.03 a	1.36± 0.04 a	1.23± 0.07 b	1.15± 0.05 c	1.10± 0.05 c	1.07± 0.05 c
T_{BM3} (10 t/ha)	1.35± 0.01 a	1.34± 0.01 a	1.29± 0.05 b	1.14± 0.02 c	1.08± 0.02 d	1.07± 0.03 d
T_{BM4} (15 t/ha)	1.27± 0.02 a	1.26± 0.00 a	1.25± 0.05 a	1.09± 0.02 b	1.03± 0.02 c	1.01± 0.01 c
T_{BM5} (20 t/ha)	1.25± 0.04 a	1.20± 0.02 b	1.18± 0.03 b	1.04± 0.01 c	1.00± 0.02 cd	0.98± 0.02 d
Available N (mg/kg)						
T_C (0 t/ha)	27.5± 2.52 f	45.5± 4.04 e	119± 8.08 d	162.75± 6.70 c	199.5± 4.04 b	211.75± 3.50a
T_{BM1} (1 t/ha)	38.25± 0.96 f	63.75± 0.96 e	143.75± 1.26 d	187.75± 0.96 c	231± 0.82 b	244.5± 1.00 a
T_{BM2} (5 t/ha)	89.25± 3.50 f	143.5± 4.04 e	254± 4.00 d	308.75± 1.50 c	361.25± 3.40 b	374.25± 4.35a
T_{BM3} (10 t/ha)	157.5± 4.04 f	250.25± 6.07 e	376.25± 6.70 d	437.5± 7.00 c	484.75± 6.70 b	504± 5.72 a
T_{BM4} (15 t/ha)	224± 5.72 f	344.75± 8.81 e	521.5± 4.04 d	588± 11.43 c	642.25± 11.95 b	658± 8.08 a
T_{BM5} (20 t/ha)	311.5± 4.04 f	444.5± 9.04 e	640.5± 13.40 d	724.5± 7.00 c	777± 8.08 b	798± 5.72 a
N mineralization (%)						
T_{BM1} (1 t/ha)	15.08 ± 1.34 e	25.60 ± 1.34 d	34.71 ± 1.76 c	37.17 ± 1.34 b	44.18± 0.81 a	45.93 ± 1.62a
T_{BM2} (5 t/ha)	17.32± 0.98 e	27.49± 1.13 d	37.87± 1.12 c	41.38± 0.42 b	45.37± 0.95 a	45.58± 1.22 a
T_{BM3} (10 t/ha)	18.23± 0.57 e	28.72± 0.94 d	36.08± 0.94 c	38.75± 0.98 b	40.01± 0.94 ab	40.99± 0.76 a
T_{BM4} (15 t/ha)	18.37± 0.53 e	27.98± 0.82 d	37.64± 0.38 c	39.90± 1.07 b	41.40± 1.12 a	41.73± 0.76 a
T_{BM5} (20 t/ha)	20.11± 0.29 e	28.26± 0.65 d	36.93± 0.95 c	39.89± 0.50 b	40.90± 0.57 a	41.52± 0.40 a

Similar letters after the values in a row are not significantly different at $p<0.05$ according to Duncan's multiple range test.

The mineralization at different incubation times (Table 8) in response to increasing dosages of blood meal showed that more than 40% mineralization was achieved within 90 days. Blood meal application at 1 t/ha produced the highest rate (46.20% ± 1.41).

For the first 45 days, blood meal application at 5 t/ha showed the maximum mineralization.

It was observed that change in the rate of N mineralization (%) was higher at the initial stages of incubation periods (15-30 days and 30-45 days) and thereafter slowed down to have minimal change at 90-120 days suggesting narrower change at greater incubation (> 120 days) time if continued.

Rahman et al. (2013) stated that the mineralization of N is influenced by the incubation period, rate of organic materials application, moisture regime and type of soil. Similar findings were also reported by other investigators (Dikinya and Mufwanzala 2010; Vel Murugan and Swarnam 2013).

Evaluation of blood meal by crop benefits

In this section, two sets of comparisons have been featured: (i) one among the blood meal treatments, and (ii) another among the manure and fertilizer types with the best blood meal dose.

Agronomic parameters

The results of statistical analysis of agronomic data and some estimated parameters among the blood meal treatments revealed that treatment T_{BM2} (blood meal- 5 t/ha) produced significantly ($p<0.05$) higher number of leaves per plant, plant height, fresh yield, and dry matter yield of *Spinacia oleracea* L. Increasing rate of blood meal gradually decreased root length and all other growth parameters. Thus, significantly ($p<0.05$) different and the lowest growth performances were observed with the highest rate of blood meal dose (T_{BM5}:20 t/ha). Studies (Biemond 1995; Richert and Salomon 1998) found that plants receiving increasing rates of N had a higher number of leaves and were reduced with higher doses of blood meal which conforms to the finding of this experiment (Table 9a). Zhang et al. (2014) in their experiment with spinach found that increasing the N fertilizer rate increased plant height and above-ground biomass yield.

The decreased plant height with higher rates of blood meal application might be due to the imbalance in the ratio of N to P caused by the prevalence of excess N in the rhizosphere. Begum et al. (2015), Cernusak et al. (2010) and Güsewell (2004) emphasized the simultaneous application of both N and P as they are the most deficient nutrients in the soil. Ullah et al. (2010), Rafiq et al. (2010) and Ahmad et al. (2009) promulgated that nitrogen enhances the production as well as the quality of grain while, Ahmad et al. (1999) proclaimed that P fertilization counterbalances the high-level of N by speeding up plant growth, enhancing grain quality by decreasing the extra growth of the vegetative parts.

Table 9a Agronomic performances of blood meal treatments

Treatments	Leaves/plant	Plant height (cm)	Root length (cm)	Fresh yield (t/ha)	Dry matter (%)	Dry matter yield (kg/ha)
T_C	7.07±0.31 bc	16.08±0.89 b	12.45±0.43 a	18.44±0.73 c	9.48±0.30 a	1747.42±72.48 c
T_{BM1}	8.07±0.70 ab	22.34±1.60 a	10.12±1.08b	29.93±0.87 b	7.57±0.41 bc	2264.17±110.75 b
T_{BM2}	8.33±0.70 a	24.77±2.61 a	8.68±0.38 c	43.14±6.75 a	7.97±0.64 b	3413.58±296.88 a
T_{BM3}	7.00±0.53 bc	21.81±1.99 a	5.28±0.44 d	24.60±2.84 b	6.71±0.12 c	1652.08±213.67 c
T_{BM4}	6.07±0.64 cd	14.07±2.77 b	4.41±0.31 d	11.52±3.02 d	7.16±0.68 bc	811.42±151.48 d
T_{BM5}	5.00±0.69 d	9.52±1.93c	3.12±0.96 e	3.02±1.77 e	7.74±1.07 bc	222.08±100.72 e

Similar letters after the values under the same column are not significantly different at $p<0.05$ according to Duncan's multiple range test

Plants grown without fertilizer addition (T_C: Control treatment) promoted significantly higher ($p<0.05$) root growth. With increasing doses of blood meal root elongation sharply declined and significantly ($p<0.05$) varied in their effect in the order of $T_C>T_{BM1}>T_{BM2} > T_{BM3} = T_{BM4} >T_{BM5}$. Fageria and Moreira (2011) in their study found that increasing nutrient supplies in the soil may also decrease root length but increase root weight in a quadratic fashion, which confirms the results of this experiment. The findings of this experiment also agree with the output of Comfort et al. (1988) study, where they stated that higher rates of application of N reduced root growth and depth of rooting in wheat and reduced root: shoot ratio in the rye (Brouwer 1966). Significantly higher ($p<0.05$) fresh yield and dry matter yield (t/ha) were obtained with 5t/ha (T_{BM2}) blood meal supplement to the soil. Among the manures, T_{BM2} significantly superseded and differed ($p<0.05$) other manures in producing the number of leaves per plant, plant height, fresh yield and dry matter yield. In the case of root elongation, T_{BM2} again performed significantly lower ($p<0.05$) than all other treatments, while, T_{CD}, T_{VC}, T_{PM}, and T_U treatments produced statistically similar effects (Table 9b). Kavvadias et al. (2013) found that nitrogen application had a significant and positive effect on fresh weight in spinach, which completely agrees with the findings of this experiment. In this experiment, both poultry manure and blood meal treatments produced a higher fresh weight of spinach because of its high N content. Badar et al. (2015) also reported the beneficial effects of organic fertilizers on the fresh weight of cowpea plants.

Table 9b Agronomic performances of manure and fertilizer types

Treatments	Leaves/plant	Plant height (cm)	Root length (cm)	Fresh yield (t/ha)	Dry matter (%)	Dry matter yield (kg/ha)
T_C	7.07±0.31 a	16.08±0.89 c	12.45±0.43 a	18.44±0.73 c	9.48±0.30 ab	1747.42±72.48 d
T_{BM2}	8.33±0.70 a	24.77±2.61 a	8.68±0.38 c	43.14±6.75 a	7.97±0.64 d	3413.58±296.88 a
T_{CD}	7.20±0.53 a	16.70±0.31 c	11.46±0.14 ab	23.98±2.00 bc	8.97±0.37 bc	2147.17±125.30 bc
T_{VC}	7.20±0.87 a	17.96±1.43 c	11.54±0.61 ab	22.65±0.83 c	8.74±0.07 c	1980.33±57.44 cd
T_{PM}	8.20±0.53 a	22.03±1.20 b	10.93±0.91 b	29.39±3.76 b	7.99±0.37 d	2343.25±235.08 b
T_U	7.80±0.87 a	16.37±0.39 c	11.64±0.36 ab	19.33±1.03 c	9.99±0.15 a	1930.50±101.79 cd

Similar letters after the values under the same column are not significantly different at $p<0.05$ according to Duncan's multiple range test.

The increase in fresh weight by organic fertilizer application has also been reported by Sarwar et al. (2008) and Manivannan et al. (2009). Abdelraouf (2016) also reported higher fresh yield and dry matter yield of spinach with N fertilizer application. Complement agreement to the results obtained in this experiment with fresh yield and dry matter yield was reported by Wang and Li (2004), they found that increasing the rate of blood meal application initially increased and declined the yield of vegetables at higher rates. Since N content in the applied fertilizer treatments was in increasing order, spinach yield was also in increasing order up to treatment T_{BM2} and declined sharply for the overabundance of N applied with T_{BM3}, T_{BM4}, and T_{BM5}. Gutiérrez-Rodríguez et al. (2013) reported that the total biomass and dry matter yield correlated with changes in total N concentration. When all blood meal-, manure- and inorganic fertilizer treatments were statistically compared together, it was observed that the spinach fresh yield (29.93 t/ha) with blood meal at a rate of 1 t/ha equaled the yield with poultry manure-10 t/ha and surpassed significantly than the yields obtained with cow dung-10 t/ha, vermicompost-10 t/ha and recommended inorganic fertilizer. Notably, the yield (43.14 t/ha) of spinach with blood meal-5 t/ha surpassed the BARC (2012) recommended yield (40±4 t/ha) and approximated the BARI (2018) recommended yield (45-50 t/ha).

Nitrogen (N) uptake and efficiency parameters

It was observed that blood meal treatments varied significantly ($p<0.05$) for nitrogen uptake, utilization, transfer and translocation (Table 10a). Among blood meal treatments, T_{BM2}, i.e., 5t/ha caused higher N-uptake, and higher transfer factor while translocation factor was found higher in T_{BM1} (1 t/ha). Lower N-utilization efficiency was achieved as it depends on dry matter yield and these two treatments produced significantly higher dry matter yield compared to T_C (Control). Comparison among fertilizer treatments showed that N uptake varied significantly ($p<0.05$) among the treatments in the order of $T_{BM2} > T_{PM} > T_{VC} = T_U = T_{CD} = T_C$ (Table 10b).

It was observed that blood meal treatment (T_{BM2}:5 t/ha) caused significantly ($p<0.05$) higher N-uptake and significantly lower N-utilization. Achieving statistically higher N-utilization with T_C treatment (Control) indicated higher N-uptake (32.9 kg/ha) compared to lower dry matter yield (1747.42 kg/ha) while achieving the lowest N-utilization indicated for a reverse phenomenon, i.e., achieving much higher dry matter yield (3413.58 kg/ha) against relatively lower N-uptake (113.37 kg/ha). A significantly higher transfer factor with T_U treatment indicated a higher proportion of N-transfer from soil to plant while a lower and different ($p<0.05$) translocation factor with T_{BM2} treatment indicated higher N-accumulation in root than translocation to shoot (Table 10b).

Table 10a Nitrogen (N) uptake and efficiency of blood meal treatments

Treatments	N uptake (kg/ha)	NUtE (kg/kg)	Transfer factor	Translocation factor
T_C	32.90±2.16 d	53.18±1.67 a	24.97±1.43 b	2.69±0.49 b
T_{BM1}	88.72±7.44 b	25.57±1.00 c	36.39±3.57a	3.87±0.25 a
T_{BM2}	113.37±12.58 a	30.17±1.19 b	29.94±6.14 ab	1.43±0.06 c
T_{BM3}	66.65±9.00 c	24.80±0.16 c	37.67±6.72 a	1.69±0.04 c
T_{BM4}	34.22±6.05 d	23.69±0.84 c	35.02±4.70 a	1.79±0.15 c
T_{BM5}	7.14±2.53 e	30.47±2.89 b	25.19±4.07 b	2.30±0.37 b

Similar letters after the values under the same column are not significantly different at $p<0.05$ according to Duncan's multiple range test.

Table 10b Nitrogen (N) uptake and efficiency parameters of manure types

Treatments	N uptake (kg/ha)	NUtE (kg/kg)	Transfer factor	Translocation factor
T_C	32.90±2.16 d	53.18±1.67 a	24.97±1.43 b	2.69±0.49 a
T_{BM2}	113.37±12.58 a	30.17±1.19 d	29.94±6.14 b	1.43±0.06 b
T_{CD}	42.74±2.39 cd	50.24±0.64 a	24.15±4.32 b	2.33±0.17 a
T_{VC}	47.25±2.16 c	41.95±1.34 b	23.98±3.80 b	2.37±0.06 a
T_{PM}	66.72±9.57 b	35.27±1.63 c	25.70±3.38 b	2.69±0.27 a
T_U	46.32±3.67 c	41.84±3.58 b	36.86±1.29 a	2.54±0.34 a

Similar letters after the values under the same column are not significantly different at $p<0.05$ according to Duncan's multiple range test.

The results of N-uptake suggested a decline with higher rates of N-input from blood meal treatments may be due to an inferior uptake with manure and inorganic fertilizer treatments other than blood meal. This outcome contradicts the results obtained by Mondal and Nad (2012). In their study, they found an increasing level of N uptake and nitrate accumulation in spinach under increasing levels of nitrogen (60,120, and 240 kg/ha).

This study found that N uptake decreased with an increasing rate of N from 5 t/ha which is equivalent to 699 kg of total N/ha. The declination of N uptake above this rate might be caused by the excess N application. The results of NUtE in this study indicated that higher efficiency in terms of yield was obtained with a lower rate of blood meal (1 t/ha and 5 t/ha) application and then NUtE lowered with the increasing rate of blood meal application. Abdelraouf (2016), Cameron et al. (2013), Canali et al. (2011) and Zhang et al. (2014) reported finding similar observations. Abdelraouf (2016) in his study on the effect of N fertilization on yield and quality of spinach found that increasing N fertilizer rates significantly decreased NUtE. He also reported obtaining higher spinach efficiency in terms of yield when a lower N fertilizer rate (56 kg N/ha) was applied. Cameron et al. (2013) in their review study on N losses from the soil/plant system reported that an increase in N fertilizer rate could lead to increased nitrogen accumulation in spinach and decreased NUtE. Under all treatments including blood meal and other fertilizers and manures, N transfer and translocation factor were >1 which indicated the accumulation of N in the plant part of spinach as described by Mugivhisa and Olowoyo (2017). Uwah et al. (2009) found an accumulation of nitrate and nitrite in spinach which showed a similarity with this study.

Conclusion

The results of the survey study, blood meal production, and characterization, blood meal incubation for N-mineralization, and crop response to blood meal application carried out under this experiment conclude that KCC produces approximately 58.6 tons of blood per annum; out of this collecta\ible fresh blood, a minimum of 10.7 tons blood meal could be produced; and the oven drying method produced the highest blood meal yield. The blood meal produced by the conventional method had significantly higher N, P, K, S, pH, and EC and significantly lower organic matter while, the blood meal produced by the oven drying method contained significantly ($p<0.05$) higher secondary nutrients (Ca and Mg), micronutrients (Fe, Cu, and Mn) and heavy metals (Cr, Pb, Cd, and Ni). The amount of available N increased with increasing dose of blood meal and the highest N mineralization was found for blood meal 5 t/ha among the blood meal treatments. The blood meal dose, 5 t/ha was the best treatment in terms of growth and yield parameters of the spinach plant. Higher than this rate suppressed the growth and yield of spinach. This dose (5 t/ha) promoted significantly higher nitrogen uptake and N-utilization efficiency. Nitrogen utilization efficiency orderly declined with increasing order of percent nitrogen content in the treatment. Comparison of the best blood meal dose with other organic manures and inorganic fertilizers also reflected that T_{BM2} (5 t/ha) superseded all other inputs in producing growth and yield of spinach. Overall, this study suggests that the application of blood meal has manifold benefits provided that the rate of application should not exceed 5 t/ha as blood meal application greater than this rate had escalated soil salinity induced stress and suppressed growth and yield of spinach plants.

This study calls for turning slaughterhouses into zero-waste, zero-pollution, and full-resource recovery units of circular agriculture, which in effect can attract investment for organic fertilizer production industries, reduce fertilizer imports, create employment opportunities and increase GDP growth.

Author’s Contribution: Md. Sanaul Islam designed and supervised the execution of the entire research work. S. M. Shahriar Zaman and Md. Nazmul Hasan Rasel conducted the experimental work and statistical analyses. S. M. Shahriar Zaman also prepared the manuscript. Md. Sanaul Islam and J.C. Joardar edited and reviewed the manuscript. All authors have read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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