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Response of Growing Male New Zealand White Rabbits to Feeding on Two Sources of Green Berseem Fodder

[1]Hamed A.A. Omer, [2]E.M. Abd El-Lateef and [2]M.F. El-Karamany

[1]Animal Production Department, National Research Centre,
33 El-Bohouth Street, P.O. Box: 12622, Dokki, Giza, Egypt
[2]Field Crops Research Department, National Research Centre,
33 El-Bohouth Street, P.O. Box: 12622, Dokki, Cairo, Egypt

Abstract: A total number of 24 male New Zealand white rabbits aged 4-5 weeks with an average body weight 676.5±64.95g were divided into two equal groups each of contains 12 rabbits. Experimental rabbits were used in a feeding trial that continuous for 49 days. Tested group rabbits were classified as Group 1: rabbits fed on green berseem fodder normal fertilize (BNF) *ad libitum* and considered as control. Group 2: rabbits fed on green berseem fodder sludge fertilize (BSF) *ad libitum*. The results showed that BSF was superior in their contents of dry matter, crude protein, nitrogen free extrct, ash, non fiber carbohydrates, gross energy, digestible energy, total digestible nutrient and digestible crude protein in comparison with BNF that that superior in their contents in crude fiber, ether extract, neutral detergent fiber, acid detergent fiber, acid detergent lignin, hemicellulose and cellulose. Heavy metal contents of two Berseem sources were in the same range. Except for crude protein, neutral detergent fiber digestibilities and digestible crude protein the other parameter were not affected (P>0.05) by the source of berseem used (BNF or BSF). All parameters of growth performance were not significantly (P<0.05) affected by the source of berseem used. Sampling time at 45 days of feeding period significantly (P<0.05) decreased heavy metal concentrations in blood plasma includes (zinc, cobalt, cadmium and lead) in comparison with that determined at 15 days. Meanwhile the others includes (iron, manganese, copper, chromium and nickel) were not significantly (P>0.05) affected by sampling time. Values of red blood cells, hematocrit, lymphocyte, monocyte and hemoglobin were not affected by the two sources of berseem, meanwhile, values of white blood cells, granulated and mean cell size were significantly (P<0.05) decreased when rabbits fed BSF comparing to that fed BNF. All values of carcass were not affected (P>0.05) by the tow sources of berseem (BNF or BSF). Heavy metal in kidneys tissues includes (iron, chromium and cadmium) were significantly (P<0.05) decreased in rabbits fed BSF. Meanwhile, it significantly (P<0.05) increased value of cobalt concentration. However, values of zinc, manganese, copper, nickel and lead concentrations were not affected. Heavy metal in liver tissues includes (iron and manganese) significantly (P<0.05) decrease in rabbits fed BSF meanwhile, cobalt concentration significantly (P<0.05) increased. But the other values of heavy metal determined were not affected (P>0.05). All values of heavy metal determined in muscles tissues were not affected (P>0.05). Heavy metal in brain tissues includes (iron and zinc) were significantly (P<0.05) decreased in rabbits fed BSF, meanwhile, copper concentarion significantly (P<0.05) increased. However the other values of heavy metal were not affected (P>0.05). It can be mentioned that rabbits can be fed on Berseem green fodder only without occurring any adverse effect on their performance, digestibility, blood, carcass.

Key words: Rabbits · Berseem · Sludge · Heavy metals · Digestibility · Performance · Carcass · Blood

INTRODUCTION

The implementation of wastewater projects in the major cities of Egypt Cairo and Alexandria will result in large quantities of sewage sludge (raw sludge, digested sludge and composted sludge) being produced and requiring disposal [1, 2]. Disposal routes must be environmentally and socially acceptable and cost-

Corresponding Author: Dr. E.M. Abd El-Lateef, Field Crops Research Department, National Research Centre, 33 El-Bohouth Street, P.O. Box: 12622, Dokki, Cairo, Egypt.

effective. Agriculture may offer the most sustainable and beneficial outlet for sewage sludge, but there are concerns about protecting the environment and human health and its practicality. The principal environmental concerns are due to the inevitable presence of potentially toxic elements (PTEs - mainly heavy metals) and human pathogens [3].

A comprehensive scientific analysis of the potential value and safety of using Cairo sludge on agricultural land as a fertilizer and soil conditioner provided the assurance that, many climatic, soil, operational, agricultural and economic factors favor agricultural use of sludge under Egyptian conditions and warm climates. Climatic and soil conditions in Egypt strongly favor a reuse option because calcareous and clay soils limit crop uptake of heavy metals and potential toxicity. Also, the reclaimed land and clay soils are deficient in Zn and Cu, as well as other essential elements which are present in sludge and required for plant growth; and the extensive sunshine exposure, high temperatures and dry conditions provide aggressive and un-favorable conditions for the survival of microbial pathogens [4-6].

Berseem (*Trifolium alexandrinum* L.) is one of the most important leguminous forages in the Mediterranean region and in the Middle-East. Berseem is an annual, sparsely hairy, erect forage legume, 30 to 80 cm high [7-9]. (Hackney *et al.,* 2007; Hannaway *et al.,* 2004; Suttie 1999

Berseem is a fast growing, high quality forage that is mainly cut and fed as green chopped forage. It is often compared to alfalfa, due to its comparable feed value. However, unlike alfalfa, it has never been reported to cause bloat. It is slightly less drought-resistant but does better on high moisture and alkalinic soils. Grazing is possible though less common than cutting. Berseem clover can also be used as green manure crop [8].

Cultivated areas reached 1.3 million ha in 2007 in Egypt [10] and 1.9 million ha in India [11]. Morocco adopted berseem in the beginning of the 20th century and 50, 000 ha were grown under irrigation in 2005 [12].

A large amount of sludge is inevitably produced in various industries, such as electroplating, tanning and mechanical manufacturing processing [13, 14]. One of the most potential of handling sludge is the application to land for improving soil fertility and structure [15-18]. However, because potentially toxic elements in sludge such as Cd, Pb, Ni and Cu pose serious threats to plant, animal and human health [19, 20], it is crucial to develop effective strategies to reduce and remove these metals from sludge [21].

Previous study have reported that ethylenediaminetetraacetic acid may pose a high risk to microorganisms and plants due to poor biodegradability and high persistence in soil and result in secondary pollution via leaching to groundwater [15, 22]. Therefore, biodegradability and minimal damage to sludge fertility are consequential considerations when searching for highly efficient WSA.

WSA derived from plant materials may be a promising alternative to these disadvantageous materials as they contain various functional groups that could bind with metal cations [23, 24]. Some studies had reported that water-soluble extracts of certain plant species, such as pineapple peel, soybean straw, Clematis brevicaudata and Coriaria nepalensis, can diminish soil nutrient loss and maintain soil organic matter [25, 26]. Furthermore, plant materials are biodegradable, widely sourced and of low–cost. Therefore, it is essential to investigate more plant materials.

Therefore, the aim of this work is to evaluate animal feeding with berseem fertilized with 6 sludge application compared with inorganic N fertilizer on forage content of Potentially Toxic metals PTEs s well as its impact on metabolic functions, blood and carcass toxicity with heavy metals of animals and the opportunities of transition of these elements to human food chain.

MATERIALS AND METHODS

This study was carried out in Co-operation work between Animal Production Department, Division of Agriculture Researches, National Research Center, Dokki, Giza, Egypt and Field Crops Department, Division of Agriculture Researches, National Research Center, Dokki, Giza, Egypt. The present work aiming to studying the response of growing male New Zealand white rabbits to eeeding on two sources of green Berseem fodder that fertlizer using two different kindes of fertilizing.

Rabbits, Feeds and Managements: A total number of 24 male New Zealand white rabbits aged 4-5 weeks with an average body weight 676.5± 64.95g were divided into two equal groups each of containing 12 rabbits.

The feeding period was extended for 49 days and the two experimental groups were classified as follow:

Group 1: Rabbits fed on green berseem fodder normal fertilize (BNF) ad libitum (*ad lib*) and considered as control.

Group 2: Rabbits fed on green berseem fodder sludge fertilize (BSF) ad libitum (*ad lib*).

Rabbits were individually housed in galvanized wire cages (30 x 35 x 40 cm). Stainless steel nipples for drinking and feeders allowing recording individual feed intake for each rabbit were supplied for each cage. Feed and water were offered *ad libitum.* All tested rabbits were kept under the same managerial conditions and were individually weighed and average feed consumption was individually weekly recorded weekly throughout the experimental period.

Digestibility Trials: At the end of the feeding trial, four rabbits from each treatment were used in digestibility trials over period of 7 days to determine the nutrient digestibility coefficients and nutritive values of the tested diets. Feces were daily collected quantitatively. Feed intake of two tested berseem used and weight of feces were daily recorded. Representative samples of feces was dried at 60 oC for 48 hrs, ground and stored for later chemical analysis. The nutritive values expressed as the total digestible nutrient (TDN) and digestible crude protein (DCP) of the experimental rations was calculated by classical method that described by Abou-Raya [27].

Blood Parameters: Four representative rabbits from each treatment were randomly chosen to take blood samples at 15, 30 and 45 days of beginning the feeding period.

Carcass Trials: Four representative rabbits from each treatment were randomly chosen and fasted for 12 hours before slaughtering according to Blasco *et al.* [28] to determine the carcass measurements. Edible offal's (Giblets) included heart, liver, kidneys, testes, lungs and spleen were removed and individually weighed. Digestive tract full and empty weights were recorded to calculate the empty body weight (EBW) by using the following equation:
Empty body weight (EBW) = Slaughter weight (SW) – digestive tract content (DTC).

where: DTC= full digestive tract weight (FDTW) - empty digestive tract weight (EDTW)

Weights of carcass, giblets and external offal's were calculated as percentages of slaughter weight (SW). Hot carcass was weighed and divided into fore limb, loin and hind limb.

Analytical Procedures: Chemical analysis of two berseem sources and feces that includes moisture, ash, crude protein (CP), crude fiber (CF) and ether extract (EE) contents were determined according to AOAC [29] methods. Crude protein determination involved the use of routine Kjeldhal nitrogen assay (N×6.25). Meanwhile, nitrogen-free extract (NFE) or carbohydrate content was determined by the difference using the following equation:

NFE content = 100 – [Moisture + CP + CF + EE + ash].

On the other hand, cell wall constituents including neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest [30] and Van Soest *et al.* [31]. Meanwhile, hemicellulose and cellulose contents were calculated by difference as follows:

Hemicellulose = NDF – ADF Cellulose = ADF - ADL.

In addition to, blood plasma parameters were analyzed using the commercial kits.

Calculations: Gross energy (Kcal/ Kg DM) was calculated according to Blaxter [32] where, each g crude protein= 5.65 Kcal, g fat = 9.40 Kcal and g (crude fiber and carbohydrate) = 4.15 Kcal.

Digestible energy (Kcal/ kg DM) was calculated according to NRC [33] where, Digestible energy (DE) = gross energy x 0.76.

Non fibrous carbohydrates (NFC) were calculated according to Calsamiglia *et al.* [34] using the following equation: NFC = 100 – {CP + EE + Ash + NDF}.

Total digestible nutrient (TDN) was calculated from data of digestibility trial according to classic method that described by Abou Raya [27].

Digestible crude protein (DCP) was calculated from data of digestibility trial according to classic method that described by Abou Raya [27].

Statistical Analysis: Data collected of feed intake, live body weight, Average daily gain, feed conversion, nutrient and cell wall constituents digestibility, nutritive values, carcass parameters and heavy metal in rabbit tissues were subjected to statistical analysis as (T Test) ore (one way) analysis of variance according to SPSS [35]. Duncan’s Multiple Range Test Duncan [36] was used to separate means when the dietary treatment effect was significant according to the following model:

$Y_{ij} = \mu + T_i + e_{ij}$ Where: Y_{ij} = observation. μ = overall mean. T_i = the effect of Berseem sources for i = 1-2, 1 = Berseem normal fertilizer (BNF) and 2 = Berseem sludge fertilizer (BSF).
e_{ij} = the experimental error.

Meanwhile, data collected of blood parameters were subjected to statistical analysis as two factors-factorial analysis of variance using the general linear model procedure of SPSS [35].

Meanwhile, Duncan's Multiple Range Test was used to examine the significance between means [36].

The following model was used as the following:
$Y_{ijk} = \mu + T_i + S_j + (TS)_{ij} + e_{ijk}$

where: Y_{ijk} = Observation. μ = the overall mean.

T_i = the effect of Berseem sources for i = 1-2, 1 = Berseem normal fertilizer (BNF) and 2 = Berseem sludge fertilizer (BSF).
S_j = the effect of sampling time for i = 1-3, 1 = first 1st sample time (ST_1) at 15 days of beginning the feeding trial, 2 = Second 2nd sample time (ST_2) at 30 days of beginning the feeding trial and 3 = third 3rd sample time (ST_3) at 45 days of beginning the feeding trial,
$(TS)_{ij}$ = the interaction between berseem sources (T) and sampling time (S).
e_{ij} = the experimental error.

RESULTS AND DISCUSSION

Chemical Analysis Green Berseem Fodder: Data of Table (1) mentioned that Berseem sludge fertilizer (BSF) was superior in their contents of dry matter (DM), crude protein (CP), nitrogen free extrct (NFE), ash, non fiber carbohydrates (NFC), gross energy (GE), digestible energy (DE), total digestible nutrient (TDN) and digestible crude protein (DCP) in comparison with Berseem normal fertilizer (BNF) that superior in their contents in the other nutrients determined that includes [crude fiber (CF), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose and cellulose]. These variations may be related to the difference in fertlizer system used that differ in their nutrients content. These results near from the results that obtained by [37-53]. who noticed that clover hay on dry matter basis (in average) contained 92.00, 87.17, 13.40, 26.03, 4.03, 43.71, 43.20, 30.06, 5.54 %, 4153 and 2661 kca/ kg DM of DM, OM, CP, CF, EE, NFE, ash, NDF, ADF and ADL, gross energy (GE) and digestible energy (DE), respectively Also, Kumar and Patel [54]) noted that values of DM, OM, CP, CF, EE, NFE and ash contents cor green berseem were 14.20, 85.09, 21.68, 18.69, 2.82, 41.90 and 14.91, for the DM, OM, CP, CF, EE, NFE and ash, respectively.

Chemical Composition of Air-Dried Sludge Samples Used in Berseem Fertilizing: Data presented in Table (2) show that the sludge used in berseem fertilizer is complied with the Egyptian code of practice for sludge reuse on the agricultural lands in Egypt.

Heavy Metal Contents of Berseem Green Fodder: As shown in Table (3) heavy metal contents of two Berseem sources that fed to the experimental rabbits were in the same range this encourage to using the sludge as an conventional source of fertlize lead to depress the costing of fertlizing of berssem consequantly occurring an improvement in farmer revenue. In this respect, Abd El-Lateef *et al*. [55] conducted trials in order to scrutinize the phytotoxicity of heavy metals to berseem, through two contrasted heavy metals behavior The first is cadmium (Cd) which represents the human dietary poison of principal concern in relation to the utilization of sewage sludge on agricultural land. This is because Cd is highly bioavailable for plant uptake and can accumulate in edible portions of crops to levels which could potentially be deleterious to humans, if consumed for long periods of time and in large quantities lead. The second element is lead (Pb) which considered as immobile element the impact of sludge Pb is unlikely to be a significant issue under Egyptian conditions because the sludges which will be used in agriculture in the future will be air-dried and will therefore be incorporated into the soil before crops are planted. Furthermore, dried sludge does not adhere to forage avoiding its likely intake by grazing livestock. The results indicate that the concentration of both elements in berseem leaves were too far from pytotoxic levels [56] which indicates and reflect in general, the application of compost improved the nutritional quality of fodder as an animal feed on calcareous soil.

Nutrients & Cell Wall Digestibility Coefficients and Nutritive Values of the Experimental Rabbits: The results of nutrient & cell wall digestibilities and nutritive values (Table 4) cleared that except for crude protein (CP), neutral detergent fiber (NDF) digestibilities and digestible crude

Table 1: Chemical analysis of two sources of green berseem (as fresh or on dry matter basis) that fed to growing New Zealand male rabbits

Item	Berseem normal fertilizer (BNF)		Berseem sludge fertilizer (BSF)	
	Fresh	DM basis	Fresh	DM basis
Moisture	84.89	-	83.38	-
Dry Matter	-	15.11	-	16.62
Chemical analysis on DM basis (%)				
Organic matter (OM)	13.52	89.47	14.85	89.35
Crude Protein (CP)	2.07	13.68	2.64	15.88
Crude fiber (CF)	4.38	29.00	4.31	25.93
Ether extract (EE)	0.29	1.92	0.27	1.62
Nitrogen free extrct (NFE)	6.78	44.87	7.63	45.92
Ash	1.59	10.53	1.77	10.65
Cell wall constituents (%)				
Neutral detergent fiber (NDF)	5.24	34.68	5.16	31.01
Acid detergent fiber (ADF)	3.08	20.36	3.03	18.20
Acid detergent lignin (ADL)	0.44	2.93	0.43	2.62
Hemicellulose[1]	2.16	14.32	2.16	12.81
Cellulose[2]	2.64	17.43	2.60	15.58
Non fiber carbohydrates (NFC)[3]	5.92	39.19	6.79	40.84
Nutritive values				
Gross energy (GE), kcal/ kg DM[4]	607	4019	670	4031
Digestible energy (DE), kcal/ kg DM[5]	461.5	3054	509	3064
Total digestible nutrient (TDN)[6]	10.42	68.94	11.49	69.16
Digestible crude protein (DCP)[7]	1.38	9.13	1.83	11.00

[1]Hemicellulos = NDF – ADF. [2]Cellulose = ADF – ADL.
[3]NFC: Non fiber carbohydrates was calculated according to Calsamiglia *et al.* [34].
[4]GE: Gross energy was calculated according to Blaxter [32].
[5]DE: Digestible energy was calculated according to NRC [33].
[6]TDN: Total digestible nutrient was calculated from data of digestibility trial according to classic method that described by Abou Raya [27].
[7]DCP: Digestible crude protein was calculated from data of digestibility trial according to classic method that described by Abou Raya [27].

Table 2: Chemical composition of air-dried sludge samples used in Berseem fertilizing (Units: ds, VS, N, P, K and Fe as %, other elements as mg kg^{-1})

Parameter		Parameter	
N (%)	1.40	Zn (mg kg^{-1})	1780
P (%)	0.50	F (mg kg^{-1})	58
K (%)	0.12	Mo (mg kg^{-1})	4.0
Mg (%)	0.50	As (mg kg^{-1})	3.44
Ca (%)	5.88	Se (mg kg^{-1})	<2.42
Fe (%)	2.59	Hg (mg kg^{-1})	2.22
Cu (mg kg^{-1})	407	Volatile solids (%)	53.3
Cd (mg kg^{-1})	2.3	Dry solids (%)	30.7
Cr (mg kg^{-1})	156		
Ni (mg kg^{-1})	48		
Pb (mg kg^{-1})	383		

Zn, Cu, Ni, Cd, Pb, Cr Mistrial Decree 222/2005* - - >2800 , >1500, >420, >39 >, 300 >, 1200 mgkg^{-1}
* Source: The Egyptian Code for Wastewater and Sludge Reuse in Agriculture (222/2005)

Table 3: Heavy metal contents of two Berseem sources that fed to the experimental rabbits

Item	Berseem normal fertilizer (BNF)	Berseem sludge fertilizer (BSF)
Iron (Fe)	38.6	38.5
Manganese (Mn)	31.1	37.9
Zinc (Zn)	18.8	20.4
Copper (Cu)	6.6	7.4
Nickel (Ni)	< 0.6	< 1.1
Cadmium (Cd)	1.2	1.4
Chromium (Cr)	0.9	0.9
Lead (Pb)	< LoD	< LoD
Cobalt (Co)	< 0.46	< 1.82

< LoD: B elow the limit of detection.
< followed by a value indicates one of the value from which the mean is drived was <loD.

Table 4: Nutrients & cell wall digestibility coefficients and nutritive values of the experimental rabbits fed two sources of berseem green fodder

Item	BNF (G_1)	BSF (G_2)	SEM
Rabbits numbers	4	4	-
Nutrient digestibility (%) of			
Dry matter (DM)	71.15	70.86	0.22
Organic matter (OM)	73.22	72.81	0.46
Crude protein (CP)	66.74^b	69.27^a	0.44
Crude fiber (CF)	49.21	48.63	0.20
Ether extract (EE)	75.12	74.73	0.35
Nitrogen-free extract (NFE)	92.51	93.27	0.31
Cell wall constituents digestibility of			
Neutral detergent fiber (NDF)	61.36^a	60.11^b	0.21
Acid detergent fiber (ADF)	57.23	56.82	0.16
Hemicellulose	67.16	66.32	0.23
Cellulose	75.11	74.36	0.22
Nutritive values (%)			
Total digestible nutrient (TDN)	68.94	69.16	0.14
Digestible crude protein (DCP)	9.13^b	11.00^a	0.28

a and b: Means in the same row having different superscripts differ significantly ($P<0.05$).
SEM: Standard error of mean. BNF: Berseem normal fertilizer. BSF: Berseem sludge fertilizer

protein (DCP) the other parameter were not affected ($P>0.05$) by the source of berseem used in rabbit feeding (BNF & BSF). These improvement in CP digestibility may be related to incrase the CP content in BSF (G_2) lead to increase CP digestibility in comparison with the control (G_1). Meanwhile increase NDF content in G_1 that fed BNF occurred an increasing in their NDF digestibility comparing to G_2 that fed BSG. Furthermore, value of digestible crude protein (DCP) was significantly ($P<0.05$) increased in G_2 that fed (BSF) compared to control (G_1) that fed BNF, this may be related to increase the DCP in BSF (11%) in comparison with the control (G_1) that recorded (9.13% of DCP) as noticed in Table (1).

These results in agreement with those found by Deshmukh and Pathak [57] who fed five adult male New Zealand White rabbits, average body weight 1556 g, singly on green berseem and they noted that mean digestibility of DM, crude protein, ether extract, crude fiber, nitrogen-free extract, acid-detergent fiber and neutral-detergent fiber was, respectively, 69.7, 80.5, 50.8, 48.9, 78.5, 58.0 and 61.3%. Mean digestible protein and total digestible nutrient values of green berseem for rabbits were 17.1 and 65.0% DM. Also, the present results are in agreement with those found by [48, 58-62] we noticed that our results were in the same trend approximately. Increased digestibility as a result of forage supplementation has been reported by [63-66]. Maximum digestibility observed at 30% level of berseem supplementation suggests that 30% level of berseem in the diet provided all the essential nutrients critical for optimal microbial activity [67], peptides, essential minerals and vitamins [68]. In addition, berseem also provided fermentable cellulose and hemicellulose, which are known to promote fiber digestion [64] by ensuring greater degree of colonization of fibrolytic bacteria [69] and fungi [70]. On the other hand, decrease nutrient digestibility coefficients as a result of 45% berseem used, as observed by Das and Singh [71] could be due to decreased mean retention time in the rumen, which means an increased rate of passage from the rumen [72] as the green forages are bulky in nature [66].

Growth Performance of the Experimental Group Rabbits: Data illustrated in Table (5) mentioned that all parameters of growth performance were not significantly ($P<0.05$) affected by the source of berseem green fodder that used, so this may be explained that it had no effect on the palatability. Values of average daily gain (ADG) were near from the other of two values. The corresponding values were 8.71 vs. 8.51 g/head/day for G_1 and G_2, respectively. These results in agreement with those found by Deshmukh and Pathak [57]. In addition to, when Omer and Badr [48] fed rabbits diet replaced berseem hay by pea straw at different levels their performance was improved.

Heavy Metal Concentrations in Blood Plasma at 15 or 45 Days of Beginning Feeding: As presented in Tables (6 & 7) data of main effect of heavy metal concentrations in blood plasma of rabbits fed two sources of berseem green fodder mentioned that heavy metal concentrations in blood plasma includes (zinc, manganese, cobalt and lead) were significantly ($P<0.05$) increased in rabbits fed berseem sludge fertilizer (BSF) in comparison with that rabbits fed Berseem normal fertilizer (BNF). Meanwhile the other heavy metal concentrations includes (iron, copper, chromium, cadmium and nickel)

Table 5: Growth performance of the experimental group rabbits fed two sources of berseem green fodder

Item	BNF (G_1)	BSF (G_2)	SEM
Rabbits numbers	12	12	-
Live Body weight (LBW), g			
Initial weight, (IW), g	665	688	64.95
Final weight (FW), g	1092	1105	87.79
Total body weight gain (TBWG), g	427	417	45.55
Experimental duration period	49 days		
Average daily gain (ADG), g	8.71	8.51	0.95
Fresh *Berseem intake (FBI), g*	313.57	290.91	25.01
Dry matter (%)	15.11	16.62	-
Feed intake (gram / head / day) of			
Dry matter intake (DMI)	47.38	48.35	3.96
Crude protein intake (CPI)	6.48	7.68	0.62
Digestible crude protein intake (DCPI)	4.33	5.32	0.43
Total digestible nutrient intake (TDNI)	32.66	33.44	2.74
Feed intake (kilo calories / head / day) of			
Gross energy intake (GEI)	190.42	194.90	15.96
Digestible energy intake (DEI)	144.70	148.14	12.13
Feed conversion expressed as (gram intake / gram gain) of			
DM	5.440	5.682	0.46
CP	0.744	0.902	0.07
DCP	0.497	0.625	0.05
TDN	3.750	3.929	0.32
Feed conversion expressed as (kilo calories intake / gram gain) of			
GE	21.86	22.90	1.86
DE	16.61	17.41	1.42

All parameters of growth performance not significant ($P<0.05$).
SEM: Standard error of mean. BNF: Berseem normal fertilizer. BSF: Berseem sludge fertilizer.

Table 6: Main effect of heavy metal concentrations in blood plasma of rabbits fed two sources of berseem green fodder

	Berseem sources			Sampling time beginning from feeding trial		
Item	BNF	BSF	SEM	At 15 days	At 45 days	SEM
Iron (FE)	0.0787	0.0640	0.0151	0.0665	0.0762	0.0151
Zinc (Zn)	0.0110^{b}	0.0630^{a}	0.0145	0.1235^{a}	0.0495^{b}	0.0145
Manganese (Mn)	0.0950^{b}	0.1565^{a}	0.0117	0.1360	0.1155	0.0117
Copper (Cu)	0.0625	0.0715	0.0032	0.0640	0.0700	0.0032
Chromium (Cr)	0.0030	0.0210	0.0047	0.0100	0.0140	0.0047
Cobalt (Co)	0.0055^{b}	0.0510^{a}	0.126	0.0545^{a}	0.0020^{b}	0.126
Cadmium (Cd)	0.0050	0.0060	0.0067	0.0075^{a}	0.0035^{b}	0.0067
Nickel (Ni)	0.0070	0.0085	0.0059	0.0075	0.0080	0.0059
Lead (Pb)	0.1815^{b}	0.2235^{a}	0.0347	0.3145^{a}	0.0905^{b}	0.0347

a and b: Means within each treatment in the same row having different superscripts differ significantly ($P<0.05$). SEM: Standard error of mean.
BNF: Berseem normal fertilizer. BSF: Berseem sludge fertilizer.

Table 7: Interaction between Berseem sources and Sampling time on heavy metal concentrations in blood plasma of rabbits

	Berseem sources							
	BNF		BSF			Significat ($P<0.05$)		
	Sampling time beginning from feeding							
Item	15 days	30 days	15 days	30 days	SEM	Source (S)	Time (T)	(SxT)
Iron (FE)	0.108	0.049	0.025	0.103	0.0151	NS	NS	NS
Zinc (Zn)	0.164^{a}	0.056^{c}	0.083^{b}	0.043^{c}	0.0145	*	*	*
Manganese (Mn)	0.106^{bc}	0.084^{c}	0.166^{a}	0.147^{ab}	0.0117	*	NS	*
Copper (Cu)	0.064	0.061	0.064	0.079	0.0032	NS	NS	NS
Chromium (Cr)	0.003	0.003	0.017	0.025	0.0047	NS	NS	NS
Cobalt (Co)	0.009^{b}	0.002^{b}	0.100^{a}	0.002^{b}	0.126	*	*	*
Cadmium (Cd)	0.007^{a}	0.003^{b}	0.008^{a}	0.004^{b}	0.0067	NS	*	*
Nickel (Ni)	0.007	0.007	0.008	0.009	0.0059	NS	NS	NS
Lead (Pb)	0.310^{a}	0.053^{c}	0.319^{a}	0.128^{b}	0.0347	*	*	*

BNF: Berseem normal fertilizer. BSF: Berseem sludge fertilizer.
a b and c: Means in the same row having different superscripts differ significantly ($P<0.05$).
SEM: Standard error of mean.

Table 8: Main effect of some blood conctituents of rabbits fed two sources of berseem green fodder

	Berseem sources			Sampling time beginning from feeding trial			
Item	BNF	BSF	SEM	At 15 days	At 30 days	At 45 days	SEM
Red blood cells (RBC's) count x 10^6 mm^3	5.47	5.50	0.196	6.00^a	5.80^a	4.65^b	0.196
White blood cells (WBC's) count x 10^3 mm^3	10.00^a	7.73^b	0.614	9.45	8.65	8.50	0.614
Hematocrit (%)	41.27	40.57	0.797	39.90	39.70	43.15	0.797
Lymphocyte (%)	75.77	81.27	3.993	80.20^a	92.35^a	63.00^b	3.993
Monocyte (%)	7.13	7.10	0.638	9.70^a	4.60^c	7.05^b	0.638
Granulated (%)	8.50^a	4.93^b	1.072	10.15^a	3.00^c	7.00^b	1.072
Hemoglobin (Hgb), g/dl^{-1}	12.73	12.37	0.337	12.55	12.60	12.50	0.337
Mean cell size	85.00^a	76.13^b	4.867	66.85^b	69.35^b	10.50^a	4.867

a b and c: Means within each treatment in the same row having different superscripts differ significantly (P<0.05). SEM: Standard error of mean.
BNF: Berseem normal fertilizer. BSF: Berseem sludge fertilizer.

Table 9: Interaction between Berseem sources and Sampling time on some blood conctituents of rabbits fed two sources of berseem green fodder

	Berseem sources									
	BNF			BSF						
	Sampling time beginning from feeding							Significat (P<0.05)		
Item	15 days (G_1)	30 days (G_2)	45 days (G_3)	15 days (G_4)	30 days (G_5)	45 days (G_6)	SEM	Source (S)	Time (T)	(SxT)
Red blood cells (RBC's) count x 10^6 mm^3	6.3^a	5.6^{abc}	4.5^c	5.7^{ab}	6.0^a	4.8b^c	0.196	NS	*	*
White blood cells (WBC's) count x 10^3 mm^3	11.5	10.1	8.4	7.4	7.2	8.6	0.614	*	NS	NS
Hematocrit (%)	41.4	39.6	42.8	38.4	39.8	43.5	0.797	NS	NS	NS
Lymphocyte (%)	76.1^{abc}	91.2^{ab}	60.0^c	84.3^a	93.5^a	66.0^{bc}	3.993	NS	*	*
Monocyte (%)	9.0^a	5.2^b	7.2^{ab}	10.4^a	4.0^b	6.9^{ab}	0.638	NS	*	*
Granulated (%)	14.9^a	3.6^{bc}	7.0^b	5.4^{bc}	2.4^c	7.0^b	1.072	*	*	*
Hemoglobin (Hgb), g/dl^{-1}	13.2	12.2	12.8	11.90	13.0	12.2	0.337	NS	NS	NS
Mean cell size	65.7^c	71.3^c	118^a	68.0^c	67.4^c	93.0^b	4.867	*	*	*

a b and c: Means in the same row having different superscripts differ significantly (P<0.05). SEM: Standard error of mean.
BNF: Berseem normal fertilizer. BSF: Berseem sludge fertilizer.

were not affected by two sources of berseem used in feeding. On the other hand sampling time at 45 days of beginning feeding period significantly (P<0.05) decreased heavy metal concentrations in blood plasma includes (zinc, cobalt, cadmium and lead) in comparison with that determined at 15 days. Moreover the other heavy metal concentrations includes (iron, manganese, copper, chromium and nickel) were not significantly (P>0.05) affected by sampling time. There were interaction between sources and sampling time on heavy metal concentrations in blood plasma that inclodues (zinc, manganese, cobalt, cadmium and lead). Meanwhile the other heavy metal concentrations includes (iron, copper, chromium and nickel) not recorded interaction between sources and sampling time.

Blood Conctituents Parameters: As shown in Tables (8 and 9) the values of red blood cells, hematocrit, lymphocyte, monocyte and hemoglobin were not affected by fedding rabbits on the two sources of berseem, meanwhile, values of white blood cells, granulated and mean cell size were significantly (P<0.05) decreased when rabbits fed BSF comparing to that fed BNF. Sampling time at 45 days of feeding significantly (P<0.05) decreased value of red blood cells, lymphocyte and monocyte, however it was significantly (P<0.05) increased the value of mean cell size comparing to sampling time at 15 and 30 days of feeding period.

On the othe hand values of white blood cells, hematocrit and hemoglobin were not significantly (P>0.05) affected by the sampling time.

There were no interaction between the sources and sampling on blood conctituents values of white blood cells, hematocrit and hemoglobin, meanwhile the other blood conctituents values includes (red blood cells, lymphocyte, monocyte, granulated and mean cell size) recorded an interactions between sources and sampling.

Carcass Characteristics of the Experimental Group: Data illustreated in Table (10) cleared that all values of carcass were not affected (P>0.05) by feeding rabbits on the tow sources of berseem used (BNF or BSF). Almost the comparing the tow values of each parameter was nearing from the other. These results were in agreement

Table 10: Carcass characteristics of the experimental group rabbits fed two sources of berseem green fodder

Item	BNF	BSF	SEM
Rabbits numbers	3	3	-
Slaughter weight (SW), g	114	1118	145.6
Non edible offals weight, g			
Blood	23	22	2.72
Fur, ears, four legs and tail	240	235	30.55
Total non edible offals	263	257	33.25
Head weight, g	63	61	7.84
Digestive tract weight, g			
Full	143	140	18.17
Empty	63	61	7.84
Content	80	79	10.33
Empty body weight (EBW)	1065	1039	135.13
Edible offals weight, g			
Heart	5	4	0.62
Liver	29	28	3.49
Kidneys	9	8	1.23
Testes	3	3	0.37
Lungs	5	6	0.62
Spleen	1	1	0.00
Total edible offals weight	52	50	6.10
Carcass weight, g			
CW_1	624	610	80.12
CW_2	687	671	87.96
CW_3	739	721	94.05
Dressing percentages (DP)			
DP1	54.50	54.56	0.096
DP2	60.00	60.02	0.113
DP3	64.54	64.49	0.042
DP4	58.59	58.71	0.112
DP5	64.51	64.58	0.108
DP6	69.39	69.39	0.046
Carcass cuts, g			
For limb	212	207	27.23
Loin	162	159	20.40
Hind limb	250	244	32.49

BNF: Berseem normal fertilizer. BSF: Berseem sludge fertilizer.
SEM: Standard error of mean. EBW = SW – digestive tract content.
CW1: Carcass weight without head and without edible offal's (heart , liver, kidneys, testes, lungs and spleen).
CW_2: Carcass weight includes head (CW_1 + head)
CW_3: Carcass weight includes head and edible offal's
DP_1: Dressing percentages calculated as (CW1 / SW * 100).
DP_2: Dressing percentages calculated as (CW2 / SW * 100).
DP_3: Dressing percentages calculated as (CW3 / SW * 100)
DP_4: Dressing percentages calculated as (CW1 / EBW * 100).
DP_5: Dressing percentages calculated as (CW2 / EBW * 100).
DP_6: Dressing percentages calculated as (CW3 / EBW * 100)

with those obtained by El-Adawy and Borhami [73]; El-Gendy *et al.* [74]; Abdel-Magid [61]; El-Medany *et al.* [62]; Omer *et al.* [44]; Omer and Badr [48]. They reported that feeding growing rabbits diets replaced clover hay with pea, chick pea or kidney beans straws, peanut hay; dried carrot processing waste or strawberry by-products had no significant differences on dressing percentages and other carcass characteristics.

Table 11: Heavy metals concentrations in different tissues of rabbits fed two sources of berseem green fodder

Item	BNF	BSF	SEM
Rabbits numbers	3	3	-
1-Kidneys tissues			
Iron (Fe)	0.584[a]	0.451[b]	0.0377
Zinc (Zn)	0.562	0.669	0.0478
Manganese (Mn)	0.282	0.270	0.0074
Copper (Cu)	0.169	0.107	0.0226
Chromium (Cr)	0.123[a]	0.063[b]	0.0163
Cobalt (Co)	0.042[b]	0.055[a]	0.0032
Cadmium (Cd)	0.320[a]	0.024[b]	0.0662
Nickel (Ni)	0.010	0.011	0.0009
Lead (Pb)	0.014	0.015	0.0004
2-Liver tissues			
Iron (FE)	0.939[a]	0.494[b]	0.1255
Zinc (Zn)	0.825	0.832	0.0631
Manganese (Mn)	0.557[a]	0.232[b]	0.0738
Copper (Cu)	0.159	0.121	0.0136
Chromium (Cr)	0.009	0.048	0.0128
Cobalt (Co)	0.031[b]	0.103[a]	0.0161
Cadmium (Cd)	0.060	0.062	0.0015
Nickel (Ni)	0.040	0.042	0.0046
Lead (Pb)	0.060	0.063	0.0038
3-Muscles tissues			
Iron (FE)	0.230	0.435	0.0879
Zinc (Zn)	0.350	0.418	0.0540
Manganese (Mn)	0.170	0.254	0.0434
Copper (Cu)	0.109	0.106	0.0073
Chromium (Cr)	0.015	0.020	0.0043
Cobalt (Co)	0.046	0.048	0.0014
Cadmium (Cd)	0.040	0.058	0.0060
Nickel (Ni)	0.009	0.010	0.0006
Lead (Pb)	0.045	0.047	0.0022
4-Brain tissues			
Iron (FE)	0.530[a]	0.401[b]	0.0307
Zinc (Zn)	0.369[a]	0.301[b]	0.0153
Manganese (Mn)	0.223	0.225	0.0196
Copper (Cu)	0.127[b]	0.139[a]	0.0036
Chromium (Cr)	0.028	0.040	0.0089
Cobalt (Co)	0.102	0.104	0.0009
Cadmium (Cd)	0.085	0.093	0.0140
Nickel (Ni)	0.053	0.054	0.0062
Lead (Pb)	0.034	0.035	0.0015

a and b: Means in the same row having different superscripts differ significantly ($P<0.05$).
SEM: Standard error of mean.
BNF: Berseem normal fertilizer.
BSF: Berseem sludge fertilizer.

Heavy Metals Concentrations in Different Carcass Tissues: Data of Table (11) mentioned that values of iron, chromium and cadmium were significantly ($P<0.05$) decreased in kidneys tissues of rabbits fed BSF comparing to that fed BNF. Meanwhile, it significantly ($P<0.05$) increased value of cobalt concentration in kidneys tissues. On the othe hand, values of zinc, manganese, copper, nickel and lead concentrations of kidneys tissues were not affected by feeding on any one of the two sources of berseem.

Also, data presented in Table (11) cleared that values of iron and manganese significantly ($P<0.05$) decrease in liver tissues of rabbits fed BSF comparing to that fed BNF, meanwhile, value of cobalt significantly ($P<0.05$) increased. But the other values of heavy metal determined in liver tissues were not affected ($P>0.05$) by the feeding on any one sources of berseem used (BNF or BSF).

In addition to results of Table (11) showed that all values of heavy metal determined in muscles tissues were not affected ($P>0.05$) by using any one sources of berseem in feeding the rabbits.

Furthermore, values of iron and zinc were significantly ($P<0.05$) decreased brain tissues of rabbits fed BSF in comparison with that received BNF, meanwhile, value of copper was significantly ($P<0.05$) increased. However the other values of heavy metal determined in brain tissues were not affected ($P>0.05$) by feeding rabbits on any one sources of berseem (Table 11).

CONCLUSION

The result demonstrated that a clear advantage of possibility of feeding rabbits on green fodder only as a conventional method of feeding used in village system with not depended on using concentrate feed mixture that used in modern rabbit farms without occurring any adverse effects on their performance. Also, source of green berseem used had no effect on values of rabbit performance, digestibility, carcass and blood. So, it can be mentioned that rabbits can be fed on Berseem green fodder only without using concentrate feed mixture in the feeding consequently the net revenue will be improved.

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