Journal of Animal Science/vol.32, No.3; 2022/pp 131-142

Effects of adding copper nanoparticles in various sources of plant and animal protein on nutrition parameters and gas production using in vitro technique

Mahnaz Haghi Jamadi[1], Jamal Seifdavati[2*], Hosein Abdi-Benemar[2] , Farzad Mirzaei Aghjehgheshlagh[2] and Reza Seyedsharifi[2]

Received: February 2, 2021 Accepted: September 21, 2021
[1]Graduate of Animal Nutrition, University of Mohaghegh Ardabili, Faculty of Agriculture and Natural Resources, Department of Animal Sciences, Ardabil, Iran.
[2] Professor of Animal Sciences, University of Mohaghegh Ardabili, Faculty of Agriculture and Natural Resources, Department of Animal Sciences, Ardabil, Iran.
[*]Corresponding author E-mail: jseifdavati@uma.ac.ir

Journal of Animal Science/vol.32 No.3/ 2022/pp 131-142
https://animalscience.tabrizu.ac.ir

DOI:10.22034/AS.2021.44766.1607

Introduction: Recent advances in nanotechnology suggest that nanoscale particles were used in medicine, dietary additives, and engineering arenas. However, their possible toxicity to people, animals, and environmental health is still unclear. The researchs showed that the ruminal fermentation of sheep with different levels of copper oxide nanoscale particles produced acetic acid and volatile fatty acid at the appropriate amount of copper oxide nanoscale particles stimulates rumen fermentation. Due to the relatively recent development of nanotechnology and limited and low researches on the special effects of copper oxide nanoscale particles on protein types, this fundamental research was to investigate the effects of adding copper oxide nanoscale particles in various types of plant and animal protein on nutrient parameters and the amount of produced gas by in vitro method.

Material and method: The amount of 100 g of copper oxide nanoscale particles was provided by Iranian agents of US Research Nanomaterial, Inc. Port Co Ltd USA. The dimensions of basic Nano-copper oxide were 40 nm, stock: US3070, in the shape of black flour and Pureness: 99%, APS: 40 nm, Color: black, Crystal Phase: single crystal, Morphology: nearly spherical, SSA: 20 m^2/g and True density: 6.4 g/ cm^3. The amount of 300 g samples were from producers of edible compounds in Ardabil province (Meshkin, Germi, and Ardabil) during 2014-2016, which include three types of plant proteins (soybean meal, canola meal, and cottonseed meal) and three types of animal protein (poultry offal meal, fish meal, and blood meal). The content of DM (Method 44-15A) and basic nutrients (the recommended procedures of AOAC measured ash-Method 08-01, CP-Method 46-06, EE with the Soxhlet method, and mineral elements were used (2005). Neutral detergent fiber (NDF) and ADF were evaluated by Van Soest *et al*. scheme (1991). The produced gas from incubated syringes at 2, 6, 12, 24, 48 and 72 h was measured by Menke and Steinggass (1988) assay. For this purpose three amounts of 0, 30, and 60 ppm of copper oxide nanoparticles was impregnated in three types of plant proteins (soybean meal, canola meal and cottonseed meal) and three types of animal protein (poultry offal meal, fish meal and blood meal) and were applied in a completely randomized plan by three replications and two-run (a total of 6 repetitions).

Results and discussion: The obtained results indicated that after 72 h, the highest of produced gas achieved for soybean meal from plant protein (58.4 mL/200 mg dry matter (DM)) and in animal

protein sources was obtained for fish meal (36.7 mL/200 mg DM). The higher digestible organic matter (DOM) and short-chain fatty acids (SCFA) were related to soybean meal, and the smaller of them was related to a blood meal. Also, the higher metabolizable energy (ME) of soybean meal for the quantities of zero were 30 and 60 ppm of copper oxide nanoscale particle, 6.48, 5.65, and 6.52 MJ/Kg DM, and the lower values of this case was found for blood meal 3.07, 3.91, and 4.01 MJ/Kg DM, respectively. The maximum and minimum amounts of microbial protein (MP) were achieved for soybean meal and blood meal 52.92 and 22.17 g/kg DOM, respectively. In some protein sources adding of copper oxide nanoscale particles are likely to directly affect the bacterial membrane, causing bacterial membrane tearing that tended to kill off bacteria. Therefore, in this study, copper oxide (containing 80% pure copper) doses of 30 ppm or mg/kg dry matter (maximum requirements) and 60 ppm or mg/kg dry matter (minimum level of possible toxicity to rumen microorganisms) were used for the individual experimental samples supplementation. It can be suggested that these levels can provide more data for future research due to the response process from the copper requirement to toxicity levels.
According to the findings of this investigation, since copper nanoscale particles have high levels of antimicrobial activity and have microbial properties, their addition to plant and animal proteins increases the bactericidal effect of some of these proteins diminishes biogas production. The addition of copper nanoscale particles due to the chemical composition of some of these proteins improved biogas production. Therefore in this study, there is a significant difference between plant and animal proteins. As observed, after adding nano copper particles, the highest amount of gas produced from soybean meal of plant origin and fish meal of animal origin was obtained.

Key words: Copper oxide nanoscale particles; Gas production; *In vitro;* Nutritional parameters; Protein types

Introduction

Copper is a trace element, important for the function of many cellular enzymes, photosynthetic processes, the exchange of hydrocarbons and proteins, as well as a part of several oxidizing enzymes, such as ascorbic acid, oxidase, and polyphenol oxidase enzymes (Machado *et al.* 2013). Recent advances in nanotechnology suggest that nanoscale particles of metal oxide can be used in various fields, from catalysts, optical and electronic materials to sensors, environmental cleanup, and biomedicine. Nanoscale particles are used in medicine, dietary additives, and engineering arenas. However, their possible toxicity to people, animals, and environmental health is still unclear (Seyedalipour *et al*. 2015). Arsenic, cadmium, copper, mercury, silver, and zinc are elements with potent antimicrobial properties. Meanwhile, the copper element is more critical in its environmental and antifungal, anti-bacterial, and even antiviral properties (Li *et al*. 2010). Copper is a rare mineral, and it is necessary as a cofactor for many enzymes that are effective in the body's biological processes (Johnson *et al*. 2014). According to published reports, copper in organic or nanoscale particles is more bioavailability than copper sulfate (Wang *et al*. 2012; Gonzales-Eguia *et al*. 2009). It is common to apply the biogas production procedure to provide an effective method for evaluating some ruminant feedstuffs. The gas test's laboratory technique is typically applied to evaluate the livestock feed (Ayasan *et al*. 2017; Menke and Stengas 1988). A simplified approach has been designed using Menke *et al*. (1979), which is functional and without delay and enables testing a large number of samples in one run. Makkar *et al*. (1995) described the new biogas production procedure's potential to consider the nutritional evaluation of feedstuff types. Recently, the biogas production system for feedstuff evaluation has been considered and approved by Singh *et al*. (2010) and Ayasan *et al*. (2017). As a result, the laboratory biogas production method is a beneficial procedure for estimating feed intake, DM, and OM digestibility, feed metabolism energy, and investigation rumen fermentation (Rezaei *et al*. 2011; Cetinkaya and Erdem 2015). Research by Mao *et al*. (2014) showed that the ruminal fermentation of sheep with different levels of copper oxide nanoscale particles produced acetic acid and volatile fatty acid at 100 to 400 μg/L levels more than other levels. As a result, the appropriate amount of copper oxide nanoscale particles stimulates rumen fermentation. A different study, (Maorong *et al*. 2008) described that the addition of copper supplements in rations improved the growth of rumen microorganisms and increased ruminal organic matter fermentability, and increased the extent of volatile fatty acids in the medium. In another study by Hongmei *et al*. (2014) was shown copper oxide nanoparticles at levels 0, 10, 20, and 30 mg/kg did not have a major influence on the forage digestibility. It was also shown that the dry matter, organic matter, acid detergent fiber (ADF), and neutral detergent fiber (NDF) digestibility of alfalfa and other local fodder was not affected by different levels of copper oxide nanoscale particles. In general, adding less than 30 mg/ kg of copper oxide nanoscale particles did not affect the digestion of ruminal materials by ruminal microbes (Arthington 2005). Due to the relatively recent development of nanotechnology and limited and low researches on the special effects of copper oxide nanoscale particles on protein types, this fundamental research was to investigate the effects of adding copper oxide nanoscale particles in various types of plant and animal protein on nutrient parameters and the amount of produced gas by in vitro method.

Materials and methods

The amount of 100 g of copper oxide nanoscale particles was provided by Iranian agents of US Research Nanomaterial, Inc. Port Co Ltd USA. The dimensions of basic Nano-copper oxide were 40 nm, stock: US3070, in the shape of black flour and Pureness: 99%, APS: 40 nm, Color: black, Crystal Phase: single crystal, Morphology: nearly spherical, SSA: 20 m^2/g and True density: 6.4 g/ cm^3.

Provision and proximate analysis of samples

The amount of 300 g samples were from producers of edible compounds in Ardabil province (Meshkin, Germi, and Ardabil) during 2014-2016, which include three types of plant proteins (soybean meal, canola meal, and cottonseed meal) and three types of animal protein (poultry offal meal, fish meal, and blood meal). The chemical composition of plant and animal protein types is shown in Table 1. The content of DM (Method 44-15A) and basic nutrients (the recommended procedures of AOAC measured ash-Method 08-01, CP-Method 46-06, EE with the Soxhlet method, and mineral elements were used (2005). Neutral detergent fiber (NDF) and ADF were evaluated by Van Soest *et al*. scheme (1991).

Biogas production

The research protocol was approved by the Animal Care and Use Committee of the Iranian Council of Animal Care released in 1995 (1995). Recommended daily dietary copper intake for cattle oscillates from 8 to 20 mg/kg of dry matter (Rezaei *et al.* 2011). An increased copper intake (greater than 40 mg Cu/kg of DM intake) is the threshold of copper toxicity level (Bidewell *et al*. 2012).

Table 1- Chemical composition of some plant and animal protein types

Protein types	DM %	CP (DM%)	EE (DM%)	Ash (DM%)	NDF (DM%)	ADF (DM%)
Plant						
Soybean meal	92.4	50.0	1.6	6.1	45.7	33.3
Canola meal	91.4	37.0	1.2	8.0	51.5	46.1
Cottonseed meal	93.0	24.0	1.4	4.7	70.6	58.4
Animal						
Poultry offal meal	94.4	55.0	31.3	7.3	48.9	34.8
Fish meal	93.6	50.0	18.1	20.0	61.2	40.6
Blood meal	70.6	59.0	1.6	5.0	55.3	33.4

DM = dry matter (percent), CP = crude protein (DM%), EE= crude fat (DM%), Ash = ash (DM%), NDF = Neutral detergent fiber (%), ADF= Acid detergent fiber (%).

Therefore, the samples of three types of plant proteins (soybean meal, canola meal, and cottonseed meal) and three types of animal protein (poultry offal meal, fish meal, and blood meal) were supplemented by 0, 30, and 60 ppm of copper oxide nanoscale. The feed samples were incubated at 39°C, and Menke and Steinggass (1988) assay was used to records the amount of gas produced at 2, 6, 12, 24, 48, and 72 h. About 200 mg DM of feed sample (milled through 2.0 mm mesh) were weighed and dumped within measured glass syringes (100 mL). Three replicates were used as blank samples. The biogas production parameters were assessed by appropriates the mean of gas volumes to the non-linear mathematical relationship 1 of the model by generalized Mitscherlich model (Palangi *et al.* 2020):

$$G=A\left(1-e^{\left(-c(t-L)-d(\sqrt{t}-\sqrt{L})\right)}\right) \quad (1)$$

Where G is the same as the total of biogas produced per hour, A is the whole of biogas produced from soluble and non-soluble fractions (mL), c is the produced biogas rate (mL per hour), d is the parameter pertaining to the variable fractional rate of degradation (mL per hour$^{0.5}$), L is the lag time, t is time of produced biogas is cumulative.

The quantity of short-chain fatty acids (SCFA) (Blummel *et al.* 1997) OM digestibility (DOM) (Menke *et al.* 1979) ME (Makkar *et al.* 1995; Menke and Stengass 1988) and microbial protein (Czerkawski 1986) were expected by the mathematical equations 2, 3, 4 and 5 as:

$$ME\ (MJ/kg\ DM) = 1.06+0.157GP+0.0084CP+0.022EE-0.0081CA \quad (2)$$

$$DOM\ (DM\ \%) = 9+0.99GP+0.0595CP+0.018CA \quad (3)$$

$$SCFA\ (mmol\ per\ 200\ mg\ DM) = 0.0222GP-0.00425 \quad (4)$$

$$MP\ (g/kg\ OMD) = [19.3\ DOM\ (kg)] \times 6.25 \quad (5)$$

Where: ME = Metabolizable energy (MJ/kg DM), GP gas is 24-hour net gas production (mL/g DM), CP is crude protein (DM %), and EE is crude fat (DM %), CA= ash in g per 100 g DM. As well as, DOM = OM digestibility (g/100 g DM), SCFA = Short-chain fatty acid (mmol), the microbial protein (MP).

The records analysis procedures and statistical model

The obtained results of the produced biogas experiment to repeated measures were analyzed by the SAS statistical software (2003). Comparing the least significant difference of means (LS Means) was done. The rest data in a completely randomized design with three repeats and three treatments were computed and the comparison of means was carried out by the Duncan test when $P \leq 0.05$. The statistical model design in equation 6 was as:

$$Y_{ij}=\mu+A_i+e_{ij} \quad (6)$$

Where: Y_{ij} is the observation, μ is the population mean, A_i is the effects of experimental treatments and eij is the residual error.

Results

Chemical composition

The test feeds chemical compositions are presented in Table 1. The maximum CP content was found for a blood meal. The greatest values of crude fat or ether extract (EE) 31.3% for poultry offal meal and the highest ash of 20% was obtained for fish meal. The maximum and lowest of NDF and ADF were achieved for cottonseed meal and soybean meal,respectively.

Biogas production

The results of accumulative produced biogas are presented in Table 2. The obtained results showed that the addition of 30 ppm of copper oxide nanoscale particles increased the amount of gas produced by soybean meal and canola meal at 48 and 72 h (P<0.05). Adding 30 and 60 ppm of copper oxide nanoscale particles significantly reduces the amount of gas produced by poultry offal meal at 12, 24, 48, and 72 h (P<0.05).

Nutritional Parameters

The effects of adding various levels of copper oxide nanoscale particles on the nutritional parameters of test feeds are shown in Table 3. Fish meal and soybean meal could not be affected by copper oxide nanoscale particles. Simultaneously, other protein types in some fermentation parameters had an important influence on the addition of copper oxide nanoscale particles. The addition of various copper oxide nanoscale particles levels increased the OMD in cottonseed meal and blood meal except for poultry offal meal, which decreased (P<0.05). The obtained results revealed that the adding of 30 and 60 ppm of copper oxide nanoscale particles lead to a major diminution in the SCFA content in poultry offal meal and canola meal. Still, this value cottonseed meal and blood meal improved considerably compared to the control group (P<0.05).

The metabolizable energy and MP of cottonseed meal and blood meal due to the addition of copper oxide nanoscale particles increased considerably compared to the control group, but these values of the poultry offal meal and canola meal decreased when copper oxide nanoscale particles were added (P<0.05).

Biogas production parameters

The fermentation or gas production parameters results of samples fitted by the generalized Mitscherlich model (Palangi *et al.* 2020) are presented in Table 4. The obtained results have shown that the addition of 60 ppm of copper oxide nanoscale particles increased the potential of produced biogas (A) in protein types of canola meal, soybean meal and cottonseed meal if the maximum potential of produced biogas in protein types were for poultry offal meal (214.6 mL) that was supplemented by 30 ppm of copper oxide nanoscale particles (P<0.05). Adding 60 ppm copper oxide nanoscale particles increased the gas production rate constants (c) in poultry offal meal, but in addition to 30 and 60 ppm in copper oxide nanoscale particles, the constant produced biogas rate diminished in cottonseed meal (P<0.05). The highest constant produced biogas rate (c) among the protein types was observed for canola meal.

Discussion

It can be assumed that the high amount of potential gas production (A) was recorded for soybean meal (290.11 vs 209.86, 96.59, 131.17, 27.50 and 25.23) in some protein sources due to degradable nitrogen was not preventive microbial activity permitting the fast-digesting sugar parts of soybean meal to be degraded according to their potential (Paya *et al*. 2007) as
well as lower ADF and NDF levels of soybean meal than the other plant protein types.

This finding was after the finds of Mansuri *et al*. (2003) and Paya *et al*. (2007), which reported high gas production for alfalfa compared with grass, wheat straw, and some tropical feeds the physicochemical properties including degradability or solubility of CP content.

Table 2- Effect of copper oxide nanoscale particles on the amount of gas produced (mL per 200 mg DM) at different times of some plant and animal protein types

Protein Types	Levels of nano-copper oxide	Incubation time					
		2 h	6 h	12 h	24 h	48 h	72 h
Soybean meal	0 ppm	4.67	17.67	32.00	45.10	55.44	58.23
	30 ppm	1.67	15.00	32.00	46.78	57.11	59.78
	60 ppm	4.67	15.337	30.67	38.66	50.66	56.67
	SEM	1.22	1.08	2.61	2.21	2.02	1.89
	P-value	0.23	0.25	0.92	0.09	0.15	0.54
Canola meal	0 ppm	0.67	8.33[a]	16.00	28.66[ab]	39.33	41.00
	30 ppm	1.00	9.63[a]	18.67	31.99[a]	43.67	47.34
	60 ppm	0.00	4.99[b]	15.33	23.99[b]	37.00	38.34
	SEM	0.51	0.87	1.97	1.67	2.09	2.52
	P-value	0.42	0.021	0.49	0.04	0.15	0.10
Cottonseed meal	0 ppm	1.00	4.50	7.00	11.33[ab]	15.42	17.75
	30 ppm	0.67	4.33	8.00	13.00[a]	16.25	17.75
	60 ppm	1.11	3.44	6.00	9.50[b]	16.22	18.73
	SEM	0.20	0.45	0.75	0.54	0.81	0.58
	P-value	0.34	0.28	0.24	0.01	0.72	0.43
Poultry offal meal	0 ppm	1.33	9.67	15.67	19.99	23.99	28.34
	30 ppm	0.67	10.17	16.00	19.33	22.33	28.67
	60 ppm	0.33	8.67	14.00	17.33	20.33	24.17
	SEM	0.58	0.50	0.61	1.39	1.50	1.78
	P-value	0.51	0.18	0.12	0.42	0.29	0.22
Fish meal	0 ppm	0.00	0.67[c]	2.33[b]	3.83[b]	5.50[b]	5.67[b]
	30 ppm	0.33	1.67[b]	4.70[ab]	4.58[b]	6.20[b]	7.67[ab]
	60 ppm	0.67	3.83[a]	7.00[a]	7.33[a]	10.50[a]	10.67[a]
	SEM	0.43	0.27	0.69	0.69	1.00	1.09
	P-value	0.58	0.01	0.01	0.03	0.03	0.05
Blood meal	0 ppm	0	1.00[b]	3.50[a]	4.50	5.08	5.75
	30 ppm	0	2.50[a]	4.50[a]	4.33	5.75	6.08
	60 ppm	0	1.00[b]	2.33[b]	3.50	5.75	6.08
	SEM	0	0.17	0.30	0.30	0.51	0.64
	P-value	0	0.01	0.01	0.12	0.59	0.91

[a,b,c] Different letters in each column represents is a significant difference ($P<0.05$)

Table 3- Effect of copper oxide nanoscale particles on parameters of nutritional some animal and plant protein types

Protein Types	Levels of nano-copper oxide	DOM (DM%)	SCFA (mmol/200mgDM)	ME (MJ/kg DM)	MP (g/kgDOM)
Soybean meal	0 ppm	56.74	0.99	8.55	68.45
	30 ppm	58.39	1.04	8.81	70.44
	60 ppm	54.87	0.85	7.54	60.75
	SEM	3.41	0.05	0.35	2.64
	P-value	0.77	0.08	0.08	0.09
Canola meal	0 ppm	39.72[ab]	0.63[ab]	5.83[ab]	47.92[ab]
	30 ppm	43.02[a]	0.71[a]	6.36[a]	51.89[a]
	60 ppm	35.10[b]	0.53[b]	5.01[b]	42.34[b]
	SEM	1.65	0.04	0.26	1.99
	P-value	0.04	0.04	0.04	0.04
Cottonseed meal	0 ppm	21.73[ab]	0.25[ab]	3.03[ab]	26.22[ab]
	30 ppm	23.38[a]	0.28[a]	3.29[a]	28.21[a]
	60 ppm	19.92[b]	0.21[b]	2.75[b]	24.03[b]
	SEM	0.53	0.01	0.08	0.64
	P-value	0.01	0.01	0.01	0.01
Poultry offal	0 ppm	32.20	0.44	5.29	38.84

meal	30 ppm	31.54	0.43	5.19	38.05
	60 ppm	29.56	0.38	4.87	35.66
	SEM	1.37	0.03	0.22	1.66
	P-value	0.42	0.42	0.42	0.42
Fish meal	0 ppm	16.13[b]	0.08[b]	2.32	19.45[b]
	30 ppm	16.87[ab]	0.09[b]	2.44	20.34[ab]
	60 ppm	19.26[a]	0.16[a]	2.74	23.24[a]
	SEM	0.71	0.02	0.13	0.86
	P-value	0.04	0.03	0.14	0.04
Blood meal	0 ppm	17.06	0.09	2.26	20.57
	30 ppm	16.89	0.09	2.23	20.34
	60 ppm	16.07	0.07	2.01	19.38
	SEM	0.30	0.01	0.04	0.36
	P-value	0.12	0.12	0.12	0.11

DOM = Digestible organic matter (DM%), SCFA = Short chain fatty acids (mmol/200mgDM), ME= Metabolizable energy (MJ/kg DM), MP = Microbial protein (g/kg DOM), SEM = Standard error of mean.
[a,b] Different letters in each column represents is a significant difference ($P<0.05$).

The lower produced biogas level happened in blood meal. Also, it had a lower produced biogas rate (c) and potential produced biogas (A). These were great in cell walls, and NPN, which have been widely stated to reduce the produced biogas rate and its amount (Guevara-Mesa *et al.* 2011; Paya *et al*. 2007). Gas production rate was similar ($P>0.01$) for the types of animal protein except for blood meal, and their average was 54-63% lower than the average of the rest animal by-products.

In some protein sources adding of copper oxide nanoscale particles are likely to directly affect the bacterial membrane, causing bacterial membrane tearing that tended to kill off bacteria. As can be seen in the microbial protein parameter of Table 4, by increasing the level and dose of nanomaterials to a toxic level of more than 30 ppm, the production of MP was reduced such as (68.45 vs 60.75 for soybean meal; 47.92 vs 42.34 for canola meal; 26.22 vs 24.03 for cottonseed meal) in plant protein. Thus, in different culture media containing copper oxide nanoscale particles, different bacteria's growth decreases (Hongmei *et al.* 2014). In research by Blummel *et al*. (1997), it was shown that there is an adverse relationship between the produced gas and the estimated SCFA with the product of microbial mass. The researchers approved no fixed partitioning between SCFA and microbial mass production (Makkar *et al*. 1995; Menke *et al*. 1979). Anti-nutritional factors such as tannin can be a problem in measuring the synthesis of raw microbial protein. In research by Cho *et al*. (2005) measured the minimum pathogen concentration for comparing the antimicrobial effects of silver nanoscale particles and platinum nanoscale particles on *Escherichia coli* and Staphylococcus bacteria. Their research showed that the silver nanoscale particles completely prevented the growth of gram-positive and harmful bacteria, but the platinum nanoscale particles did not affect the tested bacteria. It has an anti-bacterial effect on nanoscale particles, such as copper and silver, which above mentioned studies had been shown to have anti-bacterial properties of copper. In the present study, anti-bacterial properties of copper have also been observed. Mao *et al*. (2014) showed that in vitro ruminal fermentation with copper nano-oxide at levels of 100 to 400 μg/L, on the ruminal total volatile fatty acids of sheep, acetic acid was higher than the other volatile fatty acids. They concluded that the proper amount of copper oxide nanoscale particles stimulates rumen fermentation.

In another study, Maorong *et al*. (2008) described that the addition of copper supplements in the diet improved the growth of rumen microorganisms and increased the rumen organic fermentation and increased the concentration of VFAs in the medium. The findings of the investigation by Wang *et al*. (2020) showed that copper sulfate could be replaced with coated copper sulfate and the adding of coated copper sulfate enhanced milk performance and nutrient digestibility in dairy cows. In another investigation, Hongmei *et al.* (2014) reported that copper oxide nano-scale particles at 0, 10, 20, and 30 mg/kg levels had no significant effect on the forage digestibility. They also showed that the DM, OM, ADF and NDF digestibility of alfalfa was not influenced by different - copper oxide nano-scale particles and a local fodder. In total, it was concluded that adding less than 30 mg/ kg of nano-scale did not affect the digestion of ruminal materials by ruminal microbes. So that, Vázquez-Armijo *et al*. (2011) suggested depending on the dose or amount of supplementation, 21.7 ppm nano-scale particles of copper oxide have improved on the gas production and rumen fermentation, as there were positive changes in the present experiment up to 30 ppm, but at the level more than that, its harmful and toxic effects appeared. Overall, the proportion of ruminal VFA was affected by copper supplementation (Zhang *et al*. 2007), so in other studies with Cu (100 and 200 mg/day), no effect on VFA amounts (Hill and Shannon 2019; Solaiman *et al*. 2007). These consequences, and those in our search, propose that the incorporation of copper oxide nanoscale particles to the feeds can certainly affect the rumen microbes' fermenting to an extent (Ward and Spears 1993; Zhang *et al.* 2007). The supplement of Cu has increased the efficiency of fermentation, according to Solaiman *et al*. (2007) finds by diminishing the ruminal protozoa.

This is disagreeing with the review paper by Hilal *et al.* (2016), who pronounced a reduction in total nutrients digestibility of forage in cow fed by additive content an intra-ruminal Cu bolus due to its poisonous effects on rumen microbes.

It is critical that in vitro studies have recommended that the adding of increasing levels of Cu to 40 mg of Cu /kg DM did not have an adverse impact on ruminal bacteria growth (Hernández-Sánchez *et al.* 2019). Therefore, if the addition of copper nano oxide has an increasing effect on the experimental groups' parameters compared with the control group, it indicates that the requirement for this element has been met in the culture medium for the growth and microbial fermentation. Conversely, if the effect is reduced, it causes poisoning.

Table 4- Effect of copper oxide nanoscale particles on gas production parameters some animal and plant protein types by generalized Mitscherlich model (Palangi *et al.* 2020)

Protein Types	Levels of nano-copper oxide	A (mL/g DM)	c (mL per h)	d (mL per $h^{0.5}$)	L (h)
Soybean meal	0 ppm	290.11	0.08	0.074	0.34^{ab}
	30 ppm	297.76	0.09	0.0389	0.64^{a}
	60 ppm	273.26	0.06	0.058	0^{b}
	SEM	10.55	0.01	0.01	0.13
	P-value	0.32	0.21	0.18	0.022
Canola meal	0 ppm	209.86	0.06	0.076	0.58^{ab}
	30 ppm	239.10	0.06	0.106	0.52^{b}
	60 ppm	199.04	0.06	0.089	0.77^{a}
	SEM	14.84	0.01	0.01	0.06
	P-value	0.22	0.97	0.21	0.06
Cottonseed meal	0 ppm	96.59^{b}	0.04^{b}	0.159^{a}	0^{ab}
	30 ppm	89.59^{b}	0.07^{a}	0.019^{b}	0.43^{a}
	60 ppm	147.31^{a}	0.01^{c}	0.031^{b}	0^{b}
	SEM	7.01	0.01	0.01	0.34
	P-value	0.01	0.02	0.01	0.07
Poultry offal meal	0 ppm	131.17	0.09	0.065	0.17
	30 ppm	122.04	0.12	0.011	0.54
	60 ppm	101.77	0.15	0.057	0.64
	SEM	8.57	0.02	0.01	0.25
	P-value	0.12	0.23	0.10	0.44

Fish meal	0 ppm	27.50	0.11	0.068	0.71
	30 ppm	36.19	0.07	0.053	0.25
	60 ppm	50.69	0.13	0.063	0.35
	SEM	6.04	0.04	0.01	0.35
	P-value	0.08	0.65	0.63	0.63
Blood meal	0 ppm	25.23	0.18	0.038	0.87
	30 ppm	29.01	0.18	0.029	0.13
	60 ppm	35.11	0.04	0.080	0.07
	SEM	3.41	0.07	0.01	0.30
	P-value	0.19	0.28	0.07	0.19

[a,b,c] Different letters in each column represents is a significant difference ($P<0.05$).
A = Potential gas production (mL), c = Constant rate gas production (mL per hour), d = the parameter pertaining to the variable fractional rate of degradation (mL per hour$^{0.5}$), L = Lag time (hour).

If consumed in excess, it will have a harmful and toxic effect on microbes. If there is a significant increase with the control, it indicates the provision of needs and the lack of undesirable and adverse effects on microbes and the culture medium and fermentation. This is contrary to findings by Ghaffari Chanzanagh *et al*. (2018), who reported that using levels of 0, 30 and 60 ppm of ZnO nanoscale particles was not effect on produced biogas of some animal and plant protein types; however, it had a major effect on some hours of incubation of produced biogas and parameters of nutrition. As can be seen from the kinds of plant and animal protein, fish meal and poultry offal meal were not affected by different levels of copper oxide nanoscale particles in the lag phase. In contrast, other protein types significantly affected the different levels of copper oxide nanoscale particles in the lag phase. Recommended daily dietary copper intake for cattle oscillates from 8 to 20 mg/kg of dry matter and depends on several factors (Rezaei *et al.* 2011). Copper toxicity and poisoning have been defined by Bidewell *et al*. (2012) as an increased copper intake (greater than 40 mg Cu/kg of dry matter intake). Therefore, in this study, copper oxide (containing 80% pure copper) doses of 30 ppm or mg/kg dry matter (maximum requirements) and 60 ppm or mg/kg dry matter (minimum level of possible toxicity to rumen microorganisms) were used for the individual experimental samples supplementation. It can be suggested that these levels can provide more data for future research due to the response process from the copper requirement to toxicity levels.

According to the findings of this investigation, since copper nanoscale particles have high levels of antimicrobial activity and have microbial properties, their addition to plant and animal proteins increases the bactericidal effect of some of these proteins diminishes biogas production. The addition of copper nanoscale particles due to the chemical composition of some of these proteins improved biogas production. Therefore in this study, there is a significant difference between plant and animal proteins. As was observed, the highest amount of gas produced from plant proteins was reported after the addition of copper nanoscale particles from soybean meal. Among animal types, it was achieved in fish meal.

Competing Interests

There is no conflict of interest for the publication of this article.

References

AOAC (Association of Official Analytical Chemists), 2005. Official methods of analysis of the association of analytical chemists international, 18th ed. mathersburg, MD U.S.A.

Ayaşan T, Ülger İ, Baylan M, Dinçer MN, Barut H, Aykanat S, Erten HE, Ezìcì AA, Yaktubay S and Mizrak C, 2017. Determination of the nutritive value of some durum wheat (*Triticum durum* L.) varieties developed using in vitro gas production technique. Journal of the Institute of Science and Technology 7: 309-315.

Arthington JD, 2005. Effects of copper oxide bolus administration or high-level copper supplementation on forage utlization and copper status in beef cattle. Journal Animal Science 83: 2894-900.

Bidewell CA, Drew JR, Payne JH, Sayers AR, Higgins RJ and Livesey CT, 2012. Case study of copper poisoning in a British dairy herd. Veterinary Records 170:464.

Blummel M, Makkar HPS and Becker K, 1997. In vitro gas production: A technique revisited. Journal of Animal Physiology and Animal Nutrition 77: 24-34.

Cetinkaya N and Erdem F, 2015. Effect of different Juncus acutus: Maize silage rations on digestibility and rumen cellulolytic bacteria. Kafkas Universitesi Veteriner Fakultesi Dergisi 21: 499-505.

Cho KH, Park JE, Osaka T and Park SG, 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochimistry Acta 51: 956–960.

Czerkawski JW, 1986. An introduction to rumen studies. Pergamon Press, Oxford, UK.

Ghaffri Chanzanagh E, Seifdavati J, Mirzaei Aghje Gheshlagh F, Abdi Benamar H and Seyed Sharifi R, 2018. Effect of ZnO nanoparticles on in vitro gas production of some animal and plant protein types. Kafkas Universitesi Veteriner Fakultesi Dergisi 24: 25-32.

Gonzales-Eguia A, Fu CM, Lu FY and Lein TF, 2009. Effects of nano-copper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. Livestock Science 126: 122-129.

Guevara-Mesa AL, Miranda-Romero LA, Ramírez-Bribiesca JE, González-Muñoz SS, Crosby-Galvan MM, Hernández-Calva LM and Del Razo-Rodríguez OE, 2011. Protein fractions and in vitro fermentation of protein feeds for ruminants. Tropical and Subtropical Agroecosystems 14: 421-429.

Hernández-Sánchez D, Cervantes-Gómez D, Ramírez-Bribiesca JE, Cobos-Peralta M, Pinto-Ruiz R, Astigarraga L and I- Gere J, 2019. The influence of copper levels on in vitro ruminal fermentation, bacterial growth and methane production. Journal of the Science of Food and Agriculture 99: 1073–1077.

Hilal EY, Elkhairey MAE and Osman AOA, 2016. The Role of zinc, manganse and copper in rumen metabolism and immune function: A review article. Open Journal Animal Science 6: 304-324.

Hill GM and Shannon MC, 2019. Copper and zinc nutritional issues for agricultural animal production. Biological Trace Element Research 188: 148-159.

Hongmei CH, Yong G, Zhenwei D and Caixia P, 2014. Effects of nano-copper on the activities of fiber enzyme in sheep rumen. Feed Industrial 15: 12-16.

Iranian Council of Animal Care, 1995. Guide to the care and use of experimental animals, vol. 1. Isfahan University of Technology, Isfahan, Iran.(In Persian).

Johnson H, Beasley L and Macpherson N, 2014. Copper toxicity in a New Zealand dairy herd. Irish Veterinary Journal 67: 20-25.

Li B, Hwang JY, Drelich J, Popko D and Bagley S, 2010. Physical, chemical and antimicrobial characterization of copper-bearing material. JOM- Journal of the Minerals, Metals and Materials Society 62: 80-85.

Machado VS, Bicalho ML, Pereira RV, Caixeta LS, Knauer WA, Oikonomou G, Gilbert RO and Bicalho RC, 2013. Effect of an injectable trace mineral supplement containing selenium, copper, zinc and manganese on the health and production of lactating Holstein cows. Veterinary Journal 197: 451-456.

Makkar HPS, Blümmel M and Becker K, 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in vitro techniques. British Journal Nutrition 73: 897-913.

Mansuri H, Nikkhah A, Rezaeian M, Moradi Shahrbaback M and Mirhadi M, 2003. Determination of roughages degradability through In vitro gas production and nylon bag techniques. Iranian Journal of Agricultural Sciences 34: 495-507. (In Persian).

Mao Li, Zhen-Wei DAI and Cai-Xia PEI, 2014. Effect of nano-copper on volatile Fatty Acid of in vitro Rumen Fermentation in sheep. Jinshong center for Animal Husbandry and Veterinary, Collage of Animal Science and Technology, Shanxi Agricultural University. China Herbivore science.

Maorong W, Fany M, WenbinY, Yingxiang H, CHaohual M, Feny W and Yao M, 2008. Influence of copper supplementation on nitrogen metabolism and volatile fatty acid production of mixed ruminal microbial growth in continuous culture flow-through fermentors. State Key laboratory of Animal Nutrition, China Agricultural University, Beijing, 100193; 2 College of Animal science and Technology, Shanxi Agricultural University, Taigu, 03080.

Menke KH, Raab L, Salewski A, steingass H, Fritz D and Schnieider W, 1979. The estimation of the digestibility and metabolizable energy contents of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro. Journal Agricultural Science Cambridge 93: 217-222.
Menke KH and Stengass H, 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production rumen fluid. Animal Research Development 28: 7-55.
Palangi V, Macit M, and Bayat AR, 2020. Mathematical models describing disappearance of Lucerne hay in the rumen using the nylon bag technique. South African Journal of Animal Science 50:719-725.
Paya H, Taghizadeh A, Jan-mohammadi H and Moghadam GA, 2007. Nutrient digestibility and gas production of some tropical feeds used in ruminant diets estimated by the in vivo and in vitro gas production techniques. American Journal of Animal and Veterinary Sciences 2: 108-113.
Rezaei N, Salamat doust-Nobar R, Maheri Sis N, Salamatazar M, Namvari M, Goli S and Aminipour H, 2011. Effect of some plant extracts on degradability of soybean meal with gas product technique. Annals of Biological Research 2: 637-641.
SAS, 2003. SAS/STAT Software: Changes and Enhances Through Release 9.1.3. SAS Institute Inc Cary, North Carolina. USA.
Seyedalipour B, Barimani N, Dehpour Jooybari A, Hosseini SM and Oshrieh M, 2015. Histopathological evaluation of kidney and heart tissues after exposure to copper oxide nanoparticles in Mus musculus. Journal of Babol University of Medical Sciences 17: 44-50. (In Persian).
Singh B, Tomar SK and Kundu SS, 2010. In vitro gas production technique for feed evaluation. 1-115, Karnal-132 001, Haryana, India Intech Prenter andPublishers #.353, Mughal Canal Market.
Solaiman SG, Craig Jr TJ, Reddy G and Shoemaker CE, 2007. Effect of high levels of Cu supplement on growth performance, rumen fermentation, and immune responses in goat kids. Small Ruminant Research 69:115–123.
Van Soest PJ, Robertson JB and Lewis BA, 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal Dairy Science 74: 3583-3597.
Vázquez-Armijo JF, Martínez-Tinajero JJ, López D, Salem AZM and Rajo R, 2011. In vitro gas production and dry matter degradability of diets consumed by goats with or without copper and zinc supplementation. Biological Trace Element Research 144: 580-587.
Wang C, Han L, Zhang GW, Du HS, Wu ZZ, Liu Q, Guo G, Huo WJ, Zhang J, Zhang YL, Pei CX and Zhang SL, 2020. Effects of copper sulphate and coated copper sulphate addition on lactation performance, nutrient digestibility, ruminal fermentation and blood metabolites in dairy cows. British Journal of Nutrition. Cambridge Press 1: 1-9.
Wang F, Li SL, Xin J, Wang YJ, Cao ZJ, Guo FC and Wang YM, 2012. Effects of methionine hydroxy copper supplementation on lactation performance, nutrient digestibility, and blood biochemical parameters in lactating cows. Journal Dairy Science 95: 5813–5820.
Ward JD and Spears JW, 1993. Comparison of copper lysine and copper sulfate as copper types for ruminants using in vitro methods. Journal Dairy Science 76: 2994–2998.
Zhang W, Wang R, Zhu X, O-Kleemann D, Yue C and Jia Z, 2007. Effects of dietary copper on ruminal fermentation, nutrient digestibility, and fiber characteristics in cashmere goats. Asian-Australasian Journal of Animal Sciences 20: 1843–1848.

DOI:10.22034/AS.2021.44766.1607

اثرات افزودن نانو اکسید مس در منابع مختلف پروتئین گیاهی و حیوانی بر پارامترهای تغذیه ای و تولید گاز با استفاده از روش آزمایشگاهی

مهناز حقی جمادی[۱]، جمال سیف دواتی[۲*]، حسین عبدی بنمار[۲]، فرزاد میرزائی آقجه قشلاق[۲] و رضا سیدشریفی[۲]

تاریخ دریافت: ۱۳۹۹/۱۲/۱۰ تاریخ پذیرش: ۱۴۰۰/۶/۲۱

[۱]دانش آموخته کارشناسی ارشد تغذیه دام دانشکده کشاورزی و منابع طبیعی دانشگاه محقق اردبیلی
[۲] استاد گروه علوم دامی دانشکده کشاورزی و منابع طبیعی دانشگاه محقق اردبیلی
[*] مسئول مکاتبه: E-mail: jseifdavati@uma.ac.ir

چکیده

مقدمه: تحقیقات نشان داد که تخمیر شکمبه‌ای گوسفندان با مقادیر مختلف نانو اکسید مس تولید اسید استیک و اسید چرب فرار با مقدار مناسب نانو اکسید مس باعث تحریک تخمیر شکمبه می‌شود. **هدف**: این تحقیق برای بررسی اثرات نانو اکسید مس بر منابع پروتئینی مختلف بررسی اثرات نانو اکسید مس بر کنجاله های سویا، پنبه دانه، کلزا و همچنین منابع حیوانی شامل بقایای کشتارگاهی طیور، پودر ماهی و پودر خون از طریق روش‌های مختلف آزمایشگاهی انجام گرفت. **مواد و روش**: نمونه‌ها به‌طور تصادفی برای بررسی انتخاب گردیدند. گاز تولید شده از سرنگ‌ها انکوبه در ساعات مختلف اندازه گیری شد. سطوح صفر ، ۳۰ و ٦۰ قسمت در میلیون نانو اکسید مس در کنجاله‌های سویا ، کانولا ، پنبه دانه، بقایای کشتارگاهی، پودرهای ماهی و خون در یک طرح کاملاً تصادفی با سه تکرار و دو بار اجرا شد. **یافته‌ها**: نتایج نشان داد که پس از ۷۲ ساعت، بیشترین میزان گاز تولیدی از کنجاله سویا (٥٨/٤ میلی لیتر در ۲۰۰ میلی‌گرم ماده خشک) و پودر ماهی (٣٦/٧ میلی لیتر در هر ۲۰۰ میلی‌گرم) بود. بیشترین ماده آلی قابل هضم و اسیدهای چرب زنجیره کوتاه مربوط به کنجاله سویا و کوچکترین آنها مربوط به پودر خون بود. همچنین، بالاترین میزان انرژی قابل متابولیسم کنجاله سویا برای سطوح صفر ، ۳۰ و ٦۰ قسمت در میلیون نانواکسید مس، ٦/٤٨ ، ٥/٦٥ و ٦/٥٢ مگاژول در کیلوگرم ماده خشک بود و حداقل مقادیر این مورد برای پودر خون به ترتیب ٣/٠٧، ٣/٩١ و ٤/٠١ مگاژول در کیلوگرم ماده خشک دریافت شد. حداکثر و حداقل مقادیر پروتئین میکروبی به ترتیب برای کنجاله سویا و پودر خون به ترتیب ٥٢/٩٢ و ٢٢/١٧ گرم در کیلوگرم به‌دست آمد. **نتیجه گیری**: افزودن نانو اکسید مس به دلیل ترکیب شیمیایی برخی از این پروتئین‌ها ، تولید بیوگاز و پارامترهای تخمیر را در برخی از انواع منابع پروتئینی بهبود بخشد.

واژگان کلیدی: انواع پروتئین؛ تولید گاز؛ پارامترهای تغذیه ای؛ روش آزمایشگاهی؛ نانو اکسید مس