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Effect of Nickel Supplementation on Growth, Haematology, Biomarkers of Energy and Protein Metabolism of Heifers Fed Urea Based Diet

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ABSTRACT

This study was conducted to assess the effect of Nickel (Ni) supplemental effect on nutrient intake, digestibility, haematology and blood biochemical in growing cattle. Eighteen Sahiwal heifers were randomly assigned to three groups on body weight basis and fed treatment ration for 90 days. Control group were fed on basal diet without Ni and urea whereas, animals in treatment group-1 (T1) was supplemented concentrate with urea (3%) without Ni and treatment group-2 (T2) animals were supplemented with 5.0 mg Ni/kg DM/animal and concentrate containing urea (3%). The nutrient requirements of heifers were met by feeding concentrate mixture, berseem and wheat straw as per NRC (2001) guideline. Present result revealed that 5.0 mg Ni/kg DM to the diet of growing heifers had no effect on DMI, body weight gain, FCR, FCE in heifer fed urea based diet. No effects of treatments were observed on haemoglobin concentration, haematocrit value, biomarkers of lipid and protein metabolism i.e. plasma triacylglycerol, cholesterol, plasma total protein, plasma albumin and plasma globulin. The plasma glucose level was found significantly higher ($P<0.05$) in nickel supplemented group. The digestibility of CP and ADF was significantly higher ($P<0.05$) in nickel supplemented heifers fed urea based diet. The biomarkers of liver functions i.e. aspartate aminotransferase (AST), alanine aminotransferase (ALT), alanine phosphatase (ALP) and bilirubin were reported within normal physiological range. No significant differences ($P>0.05$) of Ni supplementation were observed on biomarkers of kidney function i.e. plasma creatinine and PUN. Antioxidant activity was lowered in heifers receiving 5.0 ppm Ni supplemented diets as significantly lower ($P<0.05$) FRAP and SOD values were noticed. Plasma Ca and Ni were significantly higher in Ni supplemented animals. It can be concluded that Ni supplementation at 5.0 mg Ni/kg DM showed positive effect on glucose, protein and calcium metabolism in growing cattle fed urea based diet.

KEYWORDS: Haematology, Heifers, Liver and kidney function, Nickel, Urea

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INTRODUCTION

Nickel (Ni) is recognized as a possibly essential trace element for animals. Various biochemical roles of Ni in animal body system have been found. It is an integral part of rumen urease enzyme and is required for the biosynthesis of hydrogenase enzyme. Ni has been reported to facilitating the intestinal absorption of the Fe in the animal (Shambhvi et al., 2020). It is reported to influence methane production since it is required by methanogenic bacteria in rumen for growth. Improvement in body weight gain, rumen urease activity, propionate production, antioxidant status, lipid and glucose metabolism has been reported in different species due to Ni supplementation with inconsistent results (Thamizhan, 2020). Ni play important role in the activation of enzymes, hormone action, oxidative stress, immunity, and in the regulation of the carbohydrate, protein and lipid metabolism (Kumar et al., 2022). Recent studies suggest that ruminants have an absolute requirement for Ni (McGrath et al., 2018). Ni plays a significant role in the regulation of protein metabolism by the activation of enzymes and hormones. Ni enhances recycling of nitrogen to rumen by increasing rumen epithelial urease activity. Responses to nickel supplementation have also been

higher in animals fed diets low or marginal in protein (Spears et al., 1979). A major site of action of Ni in ruminants appears to be the Ni containing enzyme urease, which is thought to play a key role in nitrogen metabolism. Nickel is poorly absorbed by the body (<10%) of intake is absorbed in the gastrointestinal tract. Nickel functions either as a structural compound or as a cofactor for enzymes in the animal body. At low concentrations, Ni specifically inhibited prolactin release, but at higher concentrations, it stimulated the release of growth hormone, thyrotropin, and adrenocorticotropic hormones from bovine pituitary (Yang and Ma, 2021). Protein source and level of crude protein are two factors that appear to influence ruminant's response to dietary nickel.

Even though the basic information regarding essentiality of Ni in growing cattle (Singh et al., 2018) pertaining to role of Ni in nutrients metabolism, enzymatic activity, antioxidant and immune status was reported recently. Since, addition of urea in commercial compounded feed is very common and about 30% of nitrogen can be supplied as NPN. But role of Ni supplementation in urea based diet was needed to be studied. Hence, present study was conducted to analyze the effect of Ni supplementation on growth, haematology and biomarkers of energy, lipid and protein metabolism of Sahiwal heifers fed urea based diet.

MATERIALS AND METHODS

A total of 18 growing female Sahiwal heifers were selected from Livestock Farm Complex (LFC), DUVASU, Mathura, India and randomly assigned into three groups (six animals in each group) on body weight and age basis. Control group were fed on basal diet without nickel and urea whereas, animals in treatment group-1 (T1) was supplemented concentrate with urea (3%) without Ni and treatment group-2 (T2) animals were supplemented Ni at the rate of 5.0 mg Ni/kg DM along with 3% urea in concentrate mixture. Ni was supplemented as nickel sulfate hexahydrate ($NiSO_4.6H_2O$, molecular weight 262.86, minimum assay purity 98%, Loba Chemie Pvt. Ltd., Mumbai) for a period of 90 days. The nutrient requirements of experimental heifers were met by feeding total mixed ration (TMR) consisted of concentrate, green berseem fodder, wheat straw in the proportion of 40:30:30 as per NRC (2001). The concentrate mixture in control group consisted of maize, barley, wheat bran, mustard oil cake, mineral mixture while in treatment groups T1 and T2 proportion of mustard cake was reduced and 3% urea as nitrogen source was incorporated as per Table 1 to make diet iso-nitrogenous. To ensure that each animal consumed the required amount of Ni, the calculated amount of Ni premix was mixed with 100 g concentrate mixture and offered to the heifers of treatment group T2. TMR was prepared daily by hand mixing and was offered at 09:00 h and 18:00 h. The animals were provided with fresh and clean drinking water free of choice twice daily at 08:00 h and 17:00 h. Experimental heifers were housed in a well-ventilated shed having the proper arrangement for individual feeding and watering without having access to the other animal's diet. Deworming of all the experimental animals was done before the start of the experiment by oral administration of Fentas bolus (Intas Pharmaceuticals Pvt. Ltd., India) at the dose level of 10 mg/kg bodyweight.

Body weight of the experimental heifers was recorded at the start of the experiment and subsequently at fortnight intervals. Heifers were weighed for two consecutive days in the morning at 0600hr before offering feeds, fodders, and water. The average of two consecutive days was considered as body weight for that fortnight and considered for ADG. The samples of feeds and fodders offered and orts left were dried in a hot air oven at 60 °C and grounded in a Wiley mill to pass a 1-mm sieve for further analysis of DM (Method 973.18c), crude protein (CP; Method 4.2.08), ether extract (EE; Method 920.85), and total ash (TA; Method 923.03) by following Association of Official Analytical Chemists procedures (AOAC, 2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF), was determined according to the procedures described by Van Soest et al. (1991). Calcium (Ca), Phosphorus (P),Copper (Cu), Ni and Fe contents in

samples of feeds and fodders were analyzed by inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA) facility at Animal Nutrition Department, DUVASU Mathura. The ingredient and nutrient com-position of TMR fed during experimental period are presented in Table1.

Table 1. Ingredients and chemical composition of TMR fed during the experimental period

Ingredient composition (%DM)	Control	T1	T2
Berseem fodder	30	30	30
Wheat straw	30	30	30
Concentrate	40	40	40
Concentrate ingredients (%DM)			
Maize	23	30	30
Barley	20	40	40
Wheat bran	20	17	17
Mustard cake	35	8	8
Urea	0	3	3
Mineral mixture	2	2	2
Chemical composition (%)			
DM	68.7	68.7	68.7
OM	91.4	91.4	91.4
EE	3.09	3.18	3.18
CP	14.0	14.4	14.4
ASH	8.59	8.55	8.55
CF	23.5	23.6	23.6
NFE	50.8	50.1	50.1
NDF	58.6	58.7	58.7
ADF	35.8	35.3	35.3
Ni	-	-	5 mg/kg

Control: basal diet without nickel and urea T1: Basal diet containing concentrate with urea (3%) without Ni; T2 Basal diet with 5 mg Ni/Kg and concentrate containing urea (3%).

Blood samples were collected before feeding and watering of heifers at 07:00 h in heparinized vacutainer tubes (BD Franklin, USA) at 0, 30, 60, and 90 days post-Ni supplementation. A fraction of blood samples were used for analysis of Hb and HIT or PCV value as per Sahli's and Wintrobe tube method, respectively. Remaining blood samples were centrifuged at 3000 rpm for 30 min to separate the plasma from packed erythrocytes. A fraction of whole blood samples were used for the analysis of superoxide dismutase (SOD; Madesh and Balasubramanian, 1998), catalase (CAT; Aebi, 1984) and FRAP (Benzie and Strain, 1999) activity. Plasma samples were stored at -20 °C until further analysis of biomarkers of liver and kidney function (AST, ALT, ALP, bilirubin, and creatinine), biomarkers of protein metabolism (total protein, albumin, globulin, and PUN), and Ni and Fe concentrations. The plasma concentration of AST, ALT, ALP, bilirubin, creatinine, total protein, albumin, and PUN was determined by an automated biochemical anal-yser (BS-120 Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co.Ltd., China) using Span Diagnostic kits (Span Diagnostic Ltd., Surat, India). Plasma globulin concentration was determined by subtracting the albumin content from total protein content. The minerals were analyzed by the inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA).

The generated data were analyzed by a mixed model for repeated measurements in Statistical Package for the Social Sciences (SPSS for windows, V21.0; SPSS Inc., Chicago, IL, USA) by using the following model:

$$Y_{ij} = \mu + H_i + T_j + e_{ij}$$

Where Y_{ij} is the dependent variable, lis the overall mean of the population, H_i is the random effect of

heifers, T_j is the fixed effect of treatment (j = 0, urea 1%, and 5.0 mg Ni/kg DM), and e_{ij} is the unexplained residual element assumed to be independent and normally distributed. Turkey honestly significant test was applied to treatment means which showed a statistically significant variation in the samples. Difference was considered significant at P<0.05.

RESULTS AND DISCUSSION

DMI (kg/day) was similar in urea supplemented or Ni supplemented animals. Though higher ADG was observed in nickel supplemented group but values were statistically similar (P=0.06). No effect of nickel supplementation was observed on body weight gain, FCR, FCE in heifer fed urea based diet (Table 2). Present findings were similar to heifers supplemented with 3.0 mg Ni/kg (Singh et al., 2018) and buffalo calves supplemented 10 mg Ni/kg (Thamizhan, 2020). Similar findings were also reported in rabbits (Bersenyi et al., 2004). However, study on lambs born as twins or triplets were randomly assigned to a synthetic, low Ni milk diet (0.03 ppm) or the basal diet with 5 ppm of Ni in a later trial. When compared to the Ni supplemented at 5 mg Ni/kg, than lambs fed the Ni deficient diet (0.03 mg Ni/kg) showed an increased mortality and decreased weight gain (Spears et al., 1978b). Similarly, adding 50 mg Ni/kg to the diet slightly increased body weight gain and enhanced feed efficiency in broiler chickens (Bersenyi et al., 2004). This indicates the role of Ni in nutrient utilization especially in protein metabolism by affecting activity of urease enzymes.

Table 2. Effect of Ni supplementation on dry matter intake and growth performance in heifers

Parameters	Treatment groups			SEM	P value		
	Control	T1	T2		Treatment (T)	Period (P)	T×P
Initial BW, Kg	137.3	137.6	137.6	11.6	1.00	-	-
Final BW, Kg	189.0	192.1	198.5	14.8	0.96	-	-
Avg BW, kg	161.9	163.1	165.6	5.17	0.75	0.95	1.00
DMI kg/day	4.12	4.05	4.21	0.13	0.79	0.86	1.00
DMI%	2.62	2.52	2.66	0.016	0.33	0.11	0.91
ADG (g/day)	574.0	605.5	675.9	26.2	0.06	0.75	0.65
FCR	8.34	7.51	7.16	0.35	0.15	0.26	0.62
FCE	0.14	0.15	0.16	0.005	0.13	0.49	0.41

P> 0.05: Non significant. SEM, standard error of mean; DMI, dry matter intake; BW, body weight; ADG, average daily gain; FCR, feed conversion ratio; FCE, feed conversion efficiency. Control: basal diet without nickel and urea T1: Basal diet containing concentrate with urea (3%) without Ni; T2 Basal diet with 5 mg Ni/Kg and concentrate containing urea (3%).

Table 3. Effect of Ni supplementation on digestibility of nutrients

Parameters	Control	T1	T2	SEM	P value
Initial BW (Kg)	180.8	184.1	188.6	14.39	0.978
Final BW (Kg)	183.5	187.2	191.9	14.56	0.976
ADG (g/day)	453.7	511.4	546.2	40.29	0.666
Digestibility coefficients (%)					
DM	58.5	62.6	64.3	1.03	0.055
OM	59.9	64.5	66.2	1.50	0.213
CP	64.4^{b}	71.6^{a}	72.0^{a}	1.18	0.001
EE	82.2	84.6	84.7	0.49	0.214
CF	53.0	56.2	58.3	0.12	0.174
NFE	65.0	64.9	66.7	1.08	0.843
NDF	57.6	58.9	61.7	0.90	0.303
ADF	48.9^{b}	54.6^{ab}	56.6^{a}	1.32	0.028

Digestibility of protein and ADF was significantly higher ($P<0.05$) in Ni supplemented animals(Table 3). Ni activates certain enzymes related to the breakdown or utilization of carbohydrate, protein and lipid. Although the apparent nutrient digestibility across seven days digestion trial was observed better in Ni supplemented heifers (T2) but values showed non significant effect ($P>0.05$). Increasing trend of digestibility was found highest with protein because Ni is needed for urease activity found in facultative rumen bacteria and this enzyme catalyzes the dietary NPN compounds into CO_2 and ammonia. Urease enzymes increase protein utilization and thus Ni supplemented group showed better growth due to better digestibility and more protein utilization. Similarly, higher urease activity, propionate concentration and decreased butyrate concentration was observed in buffalo heifers (Thamizhan, 2020). Similarly, higher nutrient digestibility was reported in heifers (Singh et al., 2018) and goat (Yousuf, 2005). Spears et al. (1978a) observed higher DM digestibility for the groups fed with higher protein concentration whereas; these attributes were not influenced by Ni supplementation. In contrary to the present findings, Bersenyi et al. (2004) found that Ni supplementation at a level of 500 mg/kg reduced the digestibility of CP by 3-4% and that of CF by 20-25% in rabbits. Improved digestibility of nutrients could be attributed to an increase in the activity of urease producing and other associated enzymes by microbial population within the rumen. Spears et al. (1979) stated that ruminants benefited more from Ni supplementation while on low protein diets as the ruminal urease enzyme converts endogenous urea to ammonia that is used for synthesis of microbial cell protein in the rumen.

In the current study, Ni supplementation at 5.0 mg/kg DM had no effect on Fe absorption or plasma levels, as evidenced by the similar plasma levels of Fe in the Ni supplemented and unsupplemented groups. As a result, blood Hb concentration and PCV percent (haematocrit value) in heifers fed with Ni were unaffected (Table 4). The current findings are similar to those of previous report of no significant alterations in hematological parameters (Hb and PCV) in Hariana heifers fed Ni up to 3 ppm (Singh et al., 2018) and in pigs showing similar WBC, RBC, Hb, and PCV on 25 ppm $NiCl_2$ supplementation in the basal diet (Spears et al., 1984). On the contrary, Chicks treated with a meal containing 2 to 15 ppb Ni

for 3 to 4 weeks had a lower hematocrit and lower O_2 consumption by liver homogenates (Nielsen and Sauberlich, 1970). Arjun et al. (2002) observed a decrease in WBC, RBC, Hb and PCV in male rats given a sub lethal dosage of Ni. This decrease could be due to Ni-induced anemia caused by hematopoietic stem cell injury. Contrarily, Thamizhan (2020) found that the values of RBC, Hb and haematocrit were the highest in Murrah buffalo calves supplemented with 10 ppm Ni in comparison to 5 ppm and 0 ppm supplemented groups. This might be due to better protein metabolism.

Table 4. Effect of Ni supplementation on haematocrit value and blood biochemical parameters

Parameters	Treatment groups			SEM	P value		
	Control	T1	T2		Treatment (T)	Period (P)	T×P
Hb, (g/dl)	9.91	9.80	9.50	0.13	0.17	0.82	0.68
PCV (%)	29.7	29.4	28.5	0.40	0.17	0.83	0.67
Glucose (mg/dl)	58.4^{b}	64.9^{ab}	72.3^{a}	2.26	<0.001	0.002	0.03
Triglyceride (mg/dl)	11.9	13.8	13.2	1.15	0.53	0.69	0.78
Cholesterol (mg/dl)	165.8	127.2	165.1	9.99	0.96	0.82	0.007
Total protein (g/L)	6.33	6.05	6.12	0.16	0.58	0.11	0.49
Albumin (g/L)	2.54^{a}	2.30^{b}	2.27^{b}	0.058	0.02	0.001	0.07
Globulin (g/L)	3.79	3.74	3.85	0.17	0.88	0.01	0.22

In the present study, plasma levels of glucose, cholesterol and triglycerides were used as biomarkers of energy and lipid metabolism while, plasma total protein, plasma albumin and globulin were used as biomarkers of protein metabolism (Table 4). The plasma glucose level was significantly higher while no difference was observed on triglyceride and cholesterol level. All biomarkers of protein metabolism are within normal physiological limit and no significant ($P>0.05$) difference were observed in Ni supplemented as well as unsupplemented heifers. In Hariana heifers, nickel supplementation up to 3 ppm had no effect on mean plasma glucose, cholesterol, or triglyceride concentrations (Singh et al., 2018). Thamizhan (2020) found blood metabolites such as plasma total protein, albumin, globulin, triglycerides, non-esterified fatty acids, creatinine, and blood urea nitrogen remain similar in Murrah buffalo calves supplemented with 10 ppm Ni compared to 5 ppm and 0 ppm supplemented groups while plasma glucose concentration was highest and total cholesterol concentration was lowest in 10 ppm supplemented calves. In present study, the higher level of glucose could be due to more propionate production. Murrah buffalo calves supplemented with 10 ppm Ni had higher urease activity, propionate concentration and decreased butyrate concentration as compared to 0 and 5 ppm groups with no significant difference in acetate concentration (Thamizhan, 2020).

Ni has an insulin-like effect on fat-cell membranes in rats, causing glucose uptake to increase and lipolysis to decrease. The Ni-induced hypo-insulinemic response was linked to the stimulation of a-2 adrenergic receptors in pancreatic islets, which modulated insulin secretion (Alverez et al., 1993). Obone et al. (1999) noticed a substantial decrease in urine volume and urine glucose levels in rats given 35 mg Ni/kg/day as nickel sulphate in drinking water for 13 weeks. Ni inhibited the facilitated diffusion of glucose at low and moderate

doses by affecting a carrier mechanism. Ni degraded intestinal wall integrity in high concentrations, resulting in paradoxical high glucose absorption or rather, and high glucose influx across the damaged wall.

In present study, biomarkers of protein metabolism (plasma total protein and plasma globulin) were unaltered in Sahiwal heifers (Table 5). Whereas, albumin was lower in urea and Ni supplemented group indicating more globulin as total protein was similar. The plasma concentrations of the biomarkers of protein metabolism (total protein, albumin, globulin and PUN) showed significantly higher ($P<0.05$) levels in Hariana heifers supplemented with 3.0 ppm Ni. Higher concentrations of these biomarkers in Ni supplemented groups showing possible role of Ni in urease activity (Singh et al., 2018).

Table 5. Effect of Ni supplementation on antioxidant status, liver and kidney function parameters

Parameters	Treatment groups			SEM	P value		
	Control	T1	T2		Treatment (T)	Period (P)	T×P
Liver and kidney function parameters							
ALT (U/L)	20.7	24.9	23.8	0.79	0.037	0.17	0.001
AST (U/L)	70.7[b]	73.8[a]	73.7[a]	0.43	0.02	0.10	0.50
ALP (U/L)	217.1	197.3	207.9	8.15	0.23	0.14	0.62
Bilirubin (mg/dl)	0.71	0.59	0.67	0.60	0.412	<0.01	0.08
Creatinine (mg/dl)	1.54	1.48	1.81	0.71	0.052	0.068	0.21
PUN (mg/dl)	9.49	9.80	8.78	0.39	0.17	0.001	0.83
Antioxidant activity parameters							
SOD (µmol MTT formazan/mg Hb)	0.45[a]	0.45[a]	0.31[b]	0.14	<0.001	<0.001	0.01
GSH-Px (ng/ml)	68.7	64.0	65.6	1.99	0.57	0.92	0.90
Catalase (nmol/ml)	66.9	65.1	64.7	0.65	0.10	0.04	0.07
FRAP (µmol/L)	1168.3[a]	1169.5[a]	1123.8[b]	6.82	0.001	<0.001	<0.001

Even though the plasma level of AST was higher ($P<0.05$) in 5.0 mg Ni/kg supplemented group indicate some affect to liver but values for all other studied biomarkers of liver functions i.e. ALT, ALP and bilirubin were reported within normal physiological range. No significant differences ($P>0.05$) of Ni supplementation were observed on biomarkers of kidney function i.e. plasma creatinine and PUN. Present findings were similar to report on heifers supplemented 5 mg Ni/kg (Singh et al., 2018). The effect of dietary Ni on plasma ALP is highly dependent on the Fe status of the animal (Nielsen and Zimmerman, 1981). The increase in plasma bilirubin level in Ni supplemented heifer may be due to defect in their metabolism and excretion, retaining it in hepatic tissues but in present finding, though values showed periodic changes but were under normal physiological level.

Plasma creatinine is an indicator of kidney function and is product of muscle creatinine metabolism (Caldeira et al., 2007). Creatinine level is elevated due to retention in the blood, it could be

used to evaluate glomerular filtration rate (Adeyemi and Akanji, 2012). Findings of elevated creatinine level in Ni group was supported by earlier report on 3.0 mg Ni/kg DM supplemented heifers showed higher plasma creatinine level (Singh et al., 2018). A Similar PUN indicates improved protein metabolism. Similar findings reported in calves (Spear et al., 1986) and heifers (Singh et al., 2018).

In present study, no significant difference (P>0.05) in the plasma GSH-Px activity and CAT activity were observed between Ni supplemented and non supplemented groups. FRAP and SOD was significantly lower (P<0.05) in heifers receiving 5.0 ppm Ni supplemented diets as SOD and FRAP reduce free radicals production (M'Bemba-Meka et al., 2005). These finding were similar to earlier study on Ni supplementation at 3 ppm in heifers (Singh et al., 2018). Thamizhan (2020) found that the values SOD and GPx remained similar, however, catalase activity was higher (P<0.05) in Murrah buffalo calves supplemented with 10 ppm Ni as compared to 5 and 0 ppm supplemented groups.

Table 6. Effect of Ni supplementation on plasma mineral profile

Parameters	Treatment groups			SEM	P value		
	Control	T1	T2		Treatment (T)	Period (P)	T×P
Ca (mg/dl)	8.68[b]	8.55[b]	10.2[a]	0.27	<0.02	0.54	0.52
P (mg/dl)	5.64	4.97	5.55	0.13	0.79	0.85	0.61
Fe (mg/L)	2.28	2.02	2.28	0.09	0.29	0.97	0.22
Cu (mg/L)	1.06	1.20	1.07	0.03	0.91	0.96	0.94
Ni (microg/L)	56.1[b]	56.2[b]	97.4[a]	5.70	<0.001	0.14	0.006

Plasma mineral profile

Nickel has been found to interact or influence the metabolism of a number of other mineral elements. In present study, dietary supplementation of Ni did not show effect on plasma concentration of P, Fe and Cu. However, plasma Ca levels were significantly higher (P<0.05) in Ni supplemented animals. This finding was similar earlier report of higher serum Ca in Ni supplemented lambs (Spears et al., 1978a). This indicates a possible role of Ni in Ca metabolism. A significantly higher plasma Ni level in Ni supplemented group (T2) was similar to earlier report on heifers Ni supplemented at 3 mg Ni/ kg (Singh et al., 2018).

CONCLUSION

From this study, it can be concluded that hematological profile showed improvement when animals were supplemented with nickel along with urea. Supplementation of nickel has increased haemoglobin and PCV. Biomarkers of lipid and protein indicated no adverse effect of treatment while there was rise in glucose level and improved protein utilization in nickel supplemented group. In present study, all liver enzymes were within normal physiological range revealing no deleterious effect on health of animals. Hence, It can be concluded that Ni supplementation at 5.0 mg Ni/kg DM positive effect on glucose, protein and calcium metabolism in growing cattle fed urea based diet.

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REFERENCES

Adeyemi, O.S. and Akanji, M.A. 2012. Psidium guajava leaf extract: effects on rat serum homeostasis and tissue morphology. Comparative Clinical Pathology. 21(4):401–407.

Aebi, H. 1984. Catalase *in vitro*. Methods in Enzymology. 105: 121-126.

Alverez C, Blade, C. and Catana, J. 1993. Alpha-2 adrenergic blockage prevents hyperglycemia and hepatic glutathione depletion in nickel injected rats. Toxicology and Applied Pharmacology. 121(1): 112–117.

AOAC. 2005. Official Methods of Analysis, 18th Edn. Association of Official Analytical Chemists, Arlington.

Arjun, J.M., Das, P.S., Day, D.S. and Das, M,K. 2002. Role of vitamin C pretreatment in reducing the blood lead level and lead induced toxic effect in erythrocyte cell membrane. Proceedings of National Academic Sciences., India, 72 (B III to IV): 313-318.

Benzie, I.F.F. and Strain, J.J. 1999. Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology. 299: 15-27.

Bersenyi, A., Fekete, S.G., Szilagyi, M., Berta, E., Zoldag, L. and Glavits R. 2004. Effects of nickel supply on the fattening performance and several biochemical parameters of broiler chickens and rabbits. Acta Veterinaria Hungarica. 52:185-197

Caldeira, R.M., Belo, A.T. and Santos, C.C. 2007. The effect of body condition score on blood metabolites and hormonal proûles in ewes. Small Ruminant Research. 68:233-241.

Kumar, M., Singh, A., Kumar, V., Kushwaha, R., Vaswani, S., Kumar, A., Shukla, P. K. and Singh, Y. 2022. Assessing essentiality of nickel in growing Hariana heifers by determining its effect on performance, nitrogen and mineral metabolism, urease activity, and endocrine biomarkers. Indian Journal of Animal Sciences. 92(11):1320–1326.

M'Bemba-Meka, P., Lemieux, N. and Chakrabarti, S.K. 2005. Role of oxidative stress, mitochondrial membrane potential, and calcium homeostasis in nickel sulfate-induced human lymphocyte death *in vitro*. Chemico Biological Interactions. 156:69–80.

Madesh, M. and Balasubramanian, K.A. 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics. 35(3): 184-188.

McGrath, J., Duval, S.M., Luis, F.M., Kindermann, T.M., Stemmler, R.T., de Gouvea, V.N., Acedo, T.S., Immig, I., Williams, S.N. and Celi, P. 2018. Nutritional strategies in ruminants: A lifetime approach. Veterinary Sciences Research Journal. 116:28–39.

Nielsen, F.H. and Sauberlich, H.E. 1970. Evidence of a possible requirement for nickel by the chick. Proceedings of the Society for Experimental Biology and Medicine. 134: 845-849.

Nielsen, F.H. and Zimmerman, T.J. 1981. Interactions among nickel, copper and iron in rats. Biological Trace Element Research. 3:83-98.

NRC. 2001. Nutrient requirements of dairy cattle, 2nd revised Edn. National Research Council, National Academy of Sciences, Washington, DC.

Obone, E., Chakrabarty, S.K., Bai, C., Malick, M.A., Lamantagne, .L. and Subramanian, K.S. 1999. Toxicity and bio-accumulation of nickel sulphate in Sprague-Dawley rats following 13 weeks of subchronic exposure. Journal of Toxicology and Environmental Health Sciences. 57: 379-401.

Pandey, R., Kumar, R., Singh, S.P., Saxena, D.K. and Srivastava, S.P. 1999. Male reproductive effect of nickel sulphate in mice. Biometals. 12:339-346.

Shambhvi, Thamizhan, P., Datt, C., Chauhan, P., Dudi, K., Thakuria, A., Singh, P. and Mani, V. 2020. Probable roles of nickel in nutrient utilization and animal performance: A review. Indian Journal of Animal Nutrition. 37(4): 299–306

Singh, A., Kumar, M., Kumar, V., Roy, D., Kushwaha, R., Vaswani, S. and Kumar, A. 2018. Effect of nickel supplementation on antioxidant status, immune characteristics, and energy and lipid metabolism in growing cattle. Biological Trace Element Research. 190: 65-75.

Spears, J.W., Hatfield, E.E. and Forbes, R.M. 1978b. Nickel depletion in the young ovine. Feed Processing. 37:404-417.

Spears, J.W., Hatfield, E.E. and Forbes, R.M. 1979. Nickel for ruminants. Influence of dietary nickel on performance and metabolic parameters. Journal of Animal. Sciences. 48:649-657.

Spears, J.W., Hatfield, E.E., Forbes, R.M. and Koenig, S.E. 1978a. Studies on the role of nickel in the ruminant. Journal of Nutrition. 108:313-320.

Spears, J.W., Jones, E.W., Samsell, L.G. and Armstrong, W.D. 1984. Effect of dietary nickel on growth, urease activity, blood parameters and tissue mineral concentrations in the neonatal pig. Journal of Nutrition. 114: 845-853.

Thamizhan, P. 2020. Influence of supplementary nickel on nutrient use efficiency, blood metabolic profile and growth in Murrah buffalo calves, M.V.Sc. Thesis, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Symposium: carbohydrate methodology, metabolism and nutritional implications in dairy cattle. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. Journal of Dairy Sciences. 74(10):3583–3597.

Yousuf, M.B. 2005. Effect of nickel supplementation on dry matter intake, nutrient digestibility and live weight change of goats fed *Panicum maximum* hay. Journal of Agricultural Research and Development. 4:23-31.