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METABOLIC AND REPRODUCTIVE RESPONSES OF EGYPTIAN BUFFALOES TO DIETARY CHROMIUM AND NIACIN SUPPLEMENTATION

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SUMMARY

An 80- days trial was conducted to investigate the effects of dietary supplemental inorganic chromium (Cr) with or without niacin on performance, blood metabolites and reproduction of Egyptian buffaloes. A total of 18 healthy buffalo heifers was randomly allotted into 3 equal groups: The first group served as control and was fed the basal diet without supplementation, heifers in the second group were fed the basal diet plus 0.75 mg inorganic Cr/ kg diet. Heifers in the third group were fed the basal diet supplemented with 0.75 mg inorganic Cr/ kg diet plus niacin (6 g/head/ day). All Heifers had free access to feed and were weighed at the onset and end of the experimental period after 24 h fasting and 16 h for water deprivation. Criteria used to measure the response were serum concentrations of glucose, cholesterol, total lipids, total protein, albumin, urea, calcium, inorganic phosphorus, ALT, AST and ALP enzymes. Moreover, serum progesterone concentration as well as fertility parameters were measured. Results indicated that chromium with or without niacin supplementations decreased ($p<0.05$) serum glucose compared to control group (67.56, 68.45 vs.79.17 mg. dl-1, respectively) and increased ($p< 0.01$) serum cholesterol (85.00, 85.63 vs. 66.25 mgl.dl-1, respectively). However, Chromium with or without niacin had no effect on nitrogen metabolism, minerals and serum enzyme activities. Meanwhile, dietary Cr with or without niacin additions significantly improved ($p<0.01$), the pregnancy rates of heifers, while, serum progesterone concentration was elevated during the first and second month of experiment. The obtained results suggest that both chromium and niacin can be used as dietary supplements for buffaloes to improve their productive and reproductive performance.

Keywords: Buffaloes, Chromium, niacin, performance, blood metabolites, fertility, progesterone.

Abbreviation key: Cr: Chromium, DMI: Dry matter intake, ALT: Alanine amino transferase enzyme, AST: Aspartic amino transferase enzyme, ALP: Alkaline phosphatase enzyme.

INTRODUCTION

Chromium (Cr) is an essential trace element for human and laboratory animals; its predominant physiological role seems to be as an integral component of the glucose tolerance factor (GTF) to potentiate the action of insulin, (Anderson, 1987). Chromium also is involved in lipid, protein and nucleic acid metabolism, (NRC, 1997). Chromium is essential for optimal insulin activity and must be complexed with certain ligands to be fully active. Niacin may become a limiting factor for absorption and / or enabling bioactivity of dietary inorganic Cr, (Chang et al. 1995). Niacin, a B vitamin, is an essential nutrient for human and non-ruminant animals and can be synthesized in the rumen or derived from dietary tryptophan in the body. In recent years, niacin has been used widely to supplement diets for high-performing ruminants, particularly dairy cattle, (Mowat et al. 1993). Available data on Cr concentration in rations for farm animals is insufficient. Moreover, even rations containing Cr. concentration satisfying the need during normal production can become sub-optimal during different stresses, such as advanced pregnancy, parturition, onset of lactation, weaning, transport, etc. (Pechova et al. 2002). However, evidence for the benefits of supplemental Cr with domestic animals including ruminants, is extremely limited. The present study was therefore conducted to investigate the effects of dietary supplemental Chromium with or without niacin on metabolic and reproductive responses of Egyptian buffalo heifers.

MATERIALS AND METHODS

1. **Animals, husbandry and experimental design:**

   The study was conducted in the experimental station belonging to the Faculty of Agriculture, El-Azhar University using 18, buffalo heifers (2 -3 years), weighing 331.0 kg on the average. Depending on rectal examination (2-3 times, 7-days apart before the start of the experiment) and evaluation of blood progestrone as described by Perera et al. (1980), all animals were confirmed as a cyclic (true anestrum). Rectal examination of those heifers that fail to show estrus usually reveals small or normal size inactive ovaries. No corpora lutea are palpable. The uterus is small and flaccid. Heifers were randomly allocated into 3 equal groups (n=6): The first group served as control and fed the basal diet only. Heifers in the second group were fed the basal diet plus 0.75 mg inorganic Cr/ kg diet. Heifers in the third group were fed the basal diet supplemented with 0.75 mg inorganic Cr/ kg diet plus niacin (6 g/head/day). The used inorganic Cr was $CrCl_3 \, 6H_2O$. The basal diet was formulated as shown in Table 1. The animals were individually fed and housed in 3 separate yards. Average DMI was 8.58 kg/ head/day. Clean fresh water was available at all times. Buffalo heifers had free access to feed and were weighed at the start as well as the end of the experimental period, (80 days) after 24 h fasting and 16 h water deprivation.

2. Metabolic and hormonal assay:

During the experimental period, blood samples were collected bi-weekly from all animals via Jugular vein puncture. Serum was separated and stored in a deep freezer at – 20°C until analysis. The measurements of serum glucose, cholesterol, total lipids, total protein, albumin, urea, calcium, inorganic phosphorus and ALT, AST and ALP enzyme activities were performed using photometric procedures and diagnostic kits, (Bio-diagnostic, Egypt.) according to Trinder (1969); Allain et al., (1974); Zollner and Kirsch (1962); Henry (1964); Drupt et al., (1974); Fawcett and Scott (1960); Gindler and King (1972); El-Merzabani et al. (1977); Maly et al. (1989a); Maly et al. (1989b); and Chromy et al. (1989), respectively.

Serum progesterone concentrations were also determined by radio-immune assay as described by Aufrere and Benson (1976) using progesterone kits (Diagnostic products corporation, USA). The sensitivity of assay was 0.05 ng / ml.

3. Reproductive traits:

Close observation and clinical examination of the buffalo heifers were performed weekly after Cr and niacin supplementation to follow up response of the heifers. All animals were checked for estrus twice- daily using intact buffalo bull. In each observation the bull was permitted to run with the females for at least 30 minutes and that come in heat were naturally bred. Pregnancy was diagnosed by rectal palpation 60 days after service. Fertility measurements including the incidence of estrus, services per conception and pregnancy rate were reported.

4. Statistical analysis:

The data were analyzed using analysis of variance (ANOVA) and chi-square procedure by stat-graphics software (1985).

RESULTS

Performance of buffalo heifers during the 80 days experimental period is presented in theTable 2. There was significant increase ($p<0.01$) in the daily gain of buffalo heifers supplemented with chromium and niacin than that in the other groups. Biochemical data are shown in Tables 3 and 4.

Blood glucose concentration was significantly lowered ($p<0.05$) in Cr with or without niacin-supplemented groups than that in the control group. The concentration of serum cholesterol was significantly higher ($p<0.01$) in the Cr with or without niacin-supplemented heifers throughout the experimental period as compared to the control . The chromium and niacin additions had no effect on total lipids, protein, albumin and urea, (Table 3). No significant differences in the enzyme activities (ALP, ALT and AST) and serum minerals (Ca and P) were observed among different groups (Table 4) The effects of Cr and niacin supplementation on reproductive performance in buffalo heifers were illustrated in Table 5. There were no significant differences ($P>0.05$) in both services per conception and incidence of estrus activity between the control and treated groups. Meanwhile, there was a clear tendency for pregnancy rate to be higher in Cr-treated groups (80.0 %) than the Cr + niacin supplemented group and the control group (60.0% and 40.0 %, respectively). Data presented in Table 6 show the overall-mean serum progesterone concentration during the first and second month of experiment was significantly ($p<0.05$) higher in Cr with or without niacin-treated groups than that in control ones.

Table 1: Ingredient and Chemical composition of the basal diet fed to buffalo heifers

Ingredient	%
Berseem hay	39.00
Concentrate mixture	59.00
Mineral and vitamin premixes*	2.00
Chemical analysis (calculated)	
CP%	13.79
TDN%	63.73
Ca%	0.74
P%	0.45

* Contained 37% limestone and mineral and vitamin mixture providing 14% Ca, 14% P, 6.24% Na, 4.0% mg. 7.500ppm Fe, 1.000ppm Zn, 1.800ppm Mn, 300ppm 1, 200ppm Cu, 40ppm Co, 1.000ppm Cl, 10ppm Se, 88.000 IU vitamin A/kg, 29.700 IU vitamin D3/kg and 150IU vitamin E/Kg.

Table 2: Effect of dietary chromium with or without niacin supplementation on Performance of buffalo heifers

Group / Item	Control	Cr	Cr+ niacin
No. of buffalo heifers	6	6	6
Live body weight, Kg			
Initial	336.8[a]	327.4[a]	329.0[a]
Final	364.0[b]	355.2[b]	368.2[a]
Body weight gain, Kg	27.20[b]	27.80[b]	39.20[a]
Daily gain, Kg	0.349[b]	0.356[b]	0.503[a]
DMI, Kg	8.580[b]	8.720[b]	9.160[a]

[a,b] values within rows with different superscripts differ significantly ($P < 0.05$).

Table 3: Effect of dietary chromium with or without niacin supplementation on blood parameters related to energy and protein metabolism in buffalo heifers

Group / Parameter	Control	Cr	Cr+ niacin
Serum glucose (mg/dl)	79.17 ± 2.75[a]	67.56 ± 3.26[b]	68.45 ± 3.71[b]
Serum cholesterol (mg/dl)	66.25 ± 7.00[b]	85.00 ± 8.85[a]	85.63 ± 9.35[a]
Serum total lipids (g/dl)	0.49 ± 0.02[a]	0.50 ± 0.02[a]	0.48 ± 0.02[a]
Serum total protein (g/l)	69.60 ± 2.37[a]	65.90 ± 2.63[a]	69.88 ± 2.42[a]
Serum albumin (g/l)	35.21 ± 1.37[a]	35.59 ± 1.71[a]	36.08 ± 2.00[a]
Serum urea (mg/dl)	59.46 ± 4.96[a]	60.27 ± 4.87[a]	61.69 ± 4.97[a]

[a,b] values within rows with different superscripts differ significantly ($P < 0.05$).

Table 4: Effect of dietary chromium with or without niacin supplementation on serum minerals and enzymes activity in buffalo heifers

Group / Parameter	Control	Cr	Cr+ niacin
Ca (mg/dl)	4.87 ± 0.48^a	5.07 ± 0.55^a	5.81 ± 0.48^a
P (mg/dl)	8.26 ± 0.45^a	8.62 ± 0.76^a	8.29 ± 0.35^a
ALP (IU/L)	25.00 ± 4.24^a	27.88 ± 3.81^a	20.67 ± 4.23^a
ALT (IU/L)	1.74 ± 0.31^a	1.87 ± 0.39^a	1.67 ± 0.40^a
AST (IU/L)	84.71 ± 6.14^a	87.26 ± 8.76^a	86.13 ± 7.26^a

The same superscript indicates no significant difference in the row at ($P < 0.05$)

Table 5: Effect of dietary chromium with or without niacin supplementation on incidence of estrus (%), services per conception (Mean±SE) and pregnancy rate (%) in buffalo heifers

Group / Period	Control (n=6)	Cr (n=6)	Cr+ niacin (n=6)
No of buffalo heifers showed estrus	5 $(83.33\%)^a$	5 $(83.33\%)^a$	5 $(83.33\%)^a$
Services per conception	1.50 ± 0.20^a	1.48 ± 0.10^a	1.45 ± 0.15^a
Pregnancy rate	2 $(40.00\%)^b$	4 $(80.00\%)^a$	3 $(60.00\%)^{ab}$

^{a,b} values within rows with different superscripts differ significantly ($P < 0.05$).

Table 6: Serum progesterone concentrations (ng/ml) in treated buffalo heifers

Group / Period	Control (n=6)	Cr (n=6)	Cr+ niacin (n=6)
Day-Zero	0.12 ± 0.037^a	0.13 ± 0.044^a	$0.15 \pm 0.0.17^a$
1st Month	0.19 ± 0.030^b	0.76 ± 0.277^a	0.68 ± 0.149^a
2nd Month	2.24 ± 0.267^b	5.97 ± 0.684^a	3.94 ± 0.992^a

^{a,b} values within rows with different superscripts differ significantly ($P < 0.05$).

DISCUSSION

Evidence for benefits of supplemental chromium and niacin for domestic animals, including ruminants, is extremely limited. There is no available data concerning the effects of supplemental Chromium and niacin on productive, reproductive performance and metabolism in buffaloes. Supplemental inorganic Cr with or without niacin improved average daily gain and feed efficiency in buffalo heifers. A similar finding was reported by Chang et al. (1995) in stressed calves. An increase in gluconeogenesis and glycogenolysis in Cr-supplemented heifers was presumed by Subiyatno et al. (1996), who demonstrated that such treatment tended to reduce insulin: glucagon. ratio. Our findings showed that Cr with or without niacin decreased ($p<0.05$) the serum glucose concentration. Such observation was in agreement with that previously reported by Mowat et al. (1993). However, Samsell and Spears (1989) and kitchalong et al. (1995) reported that there was no effect of Cr-supplementation on plasma glucose concentration in lambs. These differences in response might be due to physiological status, degree of stress, and type of nutritional stimuli. Nicotinic acid (niacin) has been demonstrated to be a component of a chromium complex potentiating insulin activity, (Chang et al. 1995). Supplemental inorganic Cr and niacin together, but not either nutrient alone, improved glucose tolerance of humans. Cr must be absorbed and complexed with certain ligands to be fully active. Nicotinic acid, glutamate, cysteine and glycine have been demonstrated to be components of a Cr complex inducing enhanced insulin sensitivity, (Mowat 1997). In human and laboratory animals, it is well known that absorption of inorganic Cr is low, less than 1% to 3%. However, natural or synthetic organic Cr complexes have been shown to be more absorbed (10 to 25%) than inorganic salts. A similar observation has been reported that inorganic Cr plus niacin together improved glucose tolerance of healthy elderly humans, (Mertz, 1993). Niacin may become a limiting factor in absorbing with or without utilizing Cr under certain conditions. It is postulated that inorganic Cr and niacin in the rumen or gastrointestinal (GI) tract with or without in the body may complex thus improving Cr absorption with or without utilization, (Chang et al., 1995). Significant increases in the serum cholesterol levels in the Cr and / or niacin-supplemented heifers were observed in the present work. Similar studies were reported in human (Anderson, 1995). We, therefore, assume that the metabolism of cholesterol in ruminants should be considered in the studies of effects of supplemental Chromium, (Brockman, 1986). On other hand, Chromium has no effect on the metabolism of nitrogen substances in our study while agrees with the finding observed by Pechova et al. (2002). The present study indicated that there were no significant differences in enzymatic activities and minerals among different groups, these changes are similar to those mentioned by Moonsie-Shgeer and Mowat (1993). Regarding the supplemental Chromium with or without niacin effect on the reproductive performance, The obtained results revealed that the pronounced effect of chromium on the pregnancy rates in buffalo heifers which had a history of reproductive problems in the form of true anestrum. Recent evidence suggested that chromium supplementation may improved sow productivity by affecting both fertility (Campbell, 1998) and fecundity, (Lindemann et al. 1995). Furthermore, Campbell (1998) showed that sows fed the diet supplemented with Cr from mating to 35 days post-mating tended to have a higher farrowing rate and the time interval to estrus from weaning was very short (7 days). He also added that the Cr-supplementation tends to improve the general health of the animal which in turn could be related to insulin senstivity and blood glucose levels during pregnancy. In the present investigation, the serum progesterone concentration was found to be higher during the first and second months after supplementation of chromium with or without niacin as compared to un-supplemented ones. The most plausible explanation for these improvements of fertility parameters may be attributed to increase in the serum cholesterol levels in the Cr and niacin-supplemented heifers which influenced directly the production of steroid hormones (Anderson, 1995 and Williams, 1994). Furthermore, low levels of cholesterol can result in a disturbance of reproductive functions (Pechova et al.2002).

Conclusions:

To the best of our knowledge, the present study is the first evidence demonstrating the beneficial effects of supplemented inorganic chromium and niacin on the productive and reproductive performance in buffalo heifers under Egyptian conditions.

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