

Original Research Article

Effect of Nano Zinc supplementation on haemato-biochemical profile in Assam Hill Goat doe

ABSTRACT

The present study was aimed to evaluate the efficiency of nano zinc (NZn) as feed supplementation on haematological and biochemical profile of Assam Hill Goat. A total of 24 numbers of 7 days post kidding doe, 2nd to 3rd parity maintained at Goat Research Station, Assam Agricultural University, Burnihat were used as experimental animal and divided into one control and three treatment groups each comprising 6 animals. Control group of animals were fed with basal diet without zinc supplementation, treatment 1 group with 25 mg NZn, treatment 2 group with 35 mg NZn and treatment 3 group with 50 mg NZn/kg concentrate mixture with basal diet for a period of three months. Blood was collected from each doe upto 3 months before treatment, fortnightly thereafter and on the day of oestrus. Results indicated that supplementation of nano zinc had no effect on haematological parameter. However, among other biochemical parameter studied, serum zinc level varied significantly ($P < 0.01$) among the groups. The serum zinc level was found to be higher in NZn-50 mg as compared to control, and NZn-25 and NZn-35 mg. Catalase and superoxide dismutase (SOD) activity were higher ($P < 0.01$) in 25 mg and 35 mg NZn/kg concentrate mixture supplemented diets as compared to the group fed with 50 mg NZn/kg concentrate mixture and control. Serum progesterone level varied significantly ($P < 0.01$) on day 84th of observations in T-2 and T-3 groups whereas serum estrogen level did not differ significantly among the group.

Keywords: *Assam hill goat; Nano zinc; catalase; superoxide dismutase; progesterone*

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INTRODUCTION

Goat rearing is a common practice in the north eastern region of India, mainly for protein source like meat, milk and allied products. Balanced nutrition by incorporation of various essential and trace minerals is the prior requirement for maintenance of good reproductive health of goat. Nutrition influences early attainment of puberty, gametogenesis, increasing conception rate and also helps to increase the number of viable offsprings produced. Although many of the nutritional influences on reproductive functions have all been well characterized, underlying mechanism for each and every nutrient is different. Zinc (Zn) as a trace mineral has an influencing role in reproduction in ruminants as it is considered to be a nutritionally imperative element due to its involvement in normal physiological process of reproduction by regulating certain reproductive hormone as well as growth and production. Oxidative stress is one of

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important cause of infertility in goat which is reported to be reduced by Zn supplementation along with feed as it plays a crucial role in synthesis of antioxidative enzymes like superoxide dismutase (SOD) [1]. Zinc is an antioxidant and its low concentrations predispose the animals to oxidative damage [2]. As zinc is not stored in our body, so a continuous dietary supplementation is required to meet the physiological needs [3]. Absorption of inorganic zinc is very less than organic, but because of its high cost, its use is limited. Therefore as an alternative to the conventional zinc source, nano zinc had been used in the present investigation to study the effect of dietary nano zinc supplementation on blood biochemical and hormonal parameters- of Assam Hill Goat doe.

MATERIALS AND METHODS

The procedure performed in the present study was ethically approved by Institutional Animal Ethics Committee (IAEC) and approval number 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/20-21/815 dated 31.07.2021 College of Veterinary Science, Khanapara, Guwahati, India. During the period of 2022-2023, twenty-four numbers of 7 days post kidding doe maintained at Goat Research Station, Assam Agricultural University, Burnihat were randomly selected and divided into one control and three treatment groups each comprising 6 animals. All the selected animals were in 2nd to 3rd parity, body weight ranging from 15-20 kg and the animals were maintained under semi-intensive system of management. For grazing the goats were let loose during day time and were supplied with 200 g of concentrate mixture along with 3-4 kg chaffed fodder daily to each animal and water supply was *ad libitum*. The does were routinely checked for health status and vaccinated against foot and mouth disease and Peste des petits ruminants (PPR) at 12 months interval and Enterotoxaemia at 6 month interval. Deworming was done at 6 months interval.

Feeding trial was conducted with the following concentration of nano zinc (Swain *et al.*, 2019) to study the effect of nano zinc on some fertility associated blood biochemical and hormonal profiles. Control group (T-0) of animals were fed with basal diet without zinc supplementation, treatment 1 group (T-1) with 25 mg NZn, treatment 2 group (T-2) with 35 mg NZn and treatment 3 group (T-3) with 50 mg NZn/kg concentrate mixture with basal diet for a period of three months. The doses of nano zinc in T-1, T-2 and T-3 were supplemented to the diets by dissolving the nano zinc in Phosphate buffer solution @ 1mg/ ml and does were fed with the help of a syringe.

Approximately 8 ml of blood was collected from each experimental animal by jugular vein puncture on the day 0 (before treatment), every fortnightly (at 14 days interval) upto 3 months of the experimental feeding and on the day of oestrus. An amount of 4ml of blood were transferred to the vacutainer containing ethylene diamine tetra acetic acid (EDTA) for the haematological studies and for determination of antioxidant. The sample vial was shaken gently to mix up the blood with the EDTA to prevent clotting. For estimation of blood biochemical parameter, another 4 ml of blood was taken in a clot activator vial. The vacutainers were kept in slanting position at room temperature for about 45 minutes for clotting. It was then transported to the laboratory and serum was separated from the coagulated blood by

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centrifugation at 3000 rpm for 10 minutes. The separated serum samples were collected in sterilized screw-capped vials. The vials were labelled and stored at -20°C for further analysis. Estimation of haematological parameters like haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) were done with the help of Autoanalyser (MS4Se, MSF J0308) by following standard procedures as per the manufacturer's protocol. Blood biochemical parameters like calcium, phosphorous, zinc, iron, catalase, superoxide dismutase, estrogen and progesterone were estimated manually by standard protocol using commercially available diagnostic kits.

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Statistical analysis of the present experimental data was done using software IBM-SPSS 20 and Microsoft Office Excel 2019. The statistical analysis was carried out by one way ANOVA, setting the probability level to $P < 0.05$, post hoc analysis of group differences was performed by LSD test.

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RESULTS AND DISCUSSION

Haematological parameter

The mean Hb, PCV, RBC and WBC level at different days of treatment and at day of oestrus with different treatment regimes are ~~were~~ presented in Table 1. The mean value of Hb, PCV, RBC and WBC did not vary significantly between different days of treatment and day of oestrus in all the groups. Results indicated that supplementation of nano zinc had no effect on haematological parameter. The haematological parameters were found in the normal ranges [4,5]. However, the author [6] observed increased ($P < 0.05$) Hb and RBC level in zinc supplemented goats as compared to control group on the 30th day of the experiment. The dietary zinc supplementation had no effect on the blood indices which might be due to insufficient amount of zinc in basal ration as reported by [4]. In addition, variations among the other studies might be due to composition of rations, zinc level and source of zinc supplied, bioavailability of zinc, animal species or breed and duration of the experimental period.

Biochemical Parameter

The level of biochemical parameters at different days of treatment and at day of oestrus with different treatment regimes are ~~were~~ presented in Table 2. The mean level of serum calcium, phosphorus and iron were did not differ significantly with different days of treatment and day of oestrus in all the groups. This might be due to amount of nano zinc fed to the animal may not be sufficient to affect on calcium, phosphorus and iron level. Similar to our observation for serum calcium, phosphorus and iron concentration, no significant effect was observed by [4] in Nubian goat kids. On the contrary, [7] recorded decreased serum calcium and phosphorus concentrations in goats supplemented with Zn from zinc oxide source in their diets. [8] observed decreased serum iron concentrations in lambs supplemented with Zn from either organic or

inorganic zinc source in their diets. As reported by [9] variations in serum calcium, phosphorous and iron level among different studies by other workers might be due to very high levels of Zn supplementation used by these investigators, which might have an antagonistic effect on their absorption.

An increasing trend for serum zinc concentration was observed among the groups from the first day to day of oestrus on the treatment groups, showing a lower level of zinc on day 0 which was before starting of treatment. The serum zinc level was found to be higher in NZn-50 mg as compared to control and NZn-25 and NZn-35 mg. The higher serum zinc levels in nano zinc supplemented groups might be due to the greater absorption of nano zinc oxide. Similar to our observation significant effect on serum zinc concentration was observed in Zandi lambs [10], Jalauni lambs [11], Angora goats [6] and in West African Dwarf goats [12]. On the contrary, some studies observed no significant effect on serum zinc level for supplementation of zinc in the diet of Angora goats [13], and dairy goats [14]. In contrast, [15] observed significantly higher plasma zinc concentration in inorganic Zn supplemented diet of Cashmere goats as compared with the control.

The mean catalase and SOD activity were observed higher in T-1 and T-2 group but the activity in T-3 was comparable to control ones. This might be due to higher dose in group T-3 which might be sufficient in preventing the ROS production. Zinc is an antioxidant and its low concentrations predispose the animals to oxidative damage [2]. Similar to our observations, [16] observed higher ($P < 0.05$) catalase and SOD activity in lambs fed with zinc supplemented diets as compared to control group. [17] observed significantly ($P < 0.05$) higher catalase and SOD activity in Ganjam sheep supplemented with zinc methionine as compared to control.

The mean estrogen level did not differ significantly among the groups with different days of observations. Progesterone level was similar in all the groups except in group T-2 and T-3 on day 84, the level was slightly higher as few animals of those groups became pregnant. [18] reported mean progesterone level in Seven Virgin Alpine goats to be 0.0-0.80 ng/ml in oestrus.

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CONCLUSION

Supplementation of nano zinc had no effect on haematological parameter and among other biochemical parameter studied, serum zinc level was found to be higher in NZn-50 mg group at different days of observation. Catalase and superoxide dismutase activity were lower in the group fed with 50 mg NZn/kg concentrate mixture and control.

REFERENCES

1. Gaafar, HMA, Basiuni MI, Ali MFE, Shitta AA, Ahamas ASE. Effect of zinc methionine supplementation on somatic cell count in milk and mastitis in Friesian cows. *Arch Zootec.* 2010;13:36-46.
2. Romanucci M, Bongiovanni I, Russo A, Capuccini S, Mechelli I, Ordeix I, et al. Oxidative stress in the pathogenesis of canine zinc responsive dermatosis. *Vet Dermatol* 2011;22:31-38.
3. Mandal GP, Das RS, Garg AK, Varshney VP, Mondal AB. Effect of zinc supplementation from inorganic and organic sources on growth and blood biochemical profile in crossbred calves. *J Anim Feed Sci.* 2008;17:147–156.
4. Elamin KM, Dafalla NA, Abdel A, Tameem EAA. Effects of Zinc Supplementation on Growth Performance and some Blood Parameters of Goat Kids in Sudan. *IJPAB.* 2013;1: 1-8.
5. Swain PS, Rao SBN, Rajendran, Poornachandra KT, Lokeshae, Dhinesh KR. Effect of Nanozinc Supplementation on Haematological and Blood Biochemical Profiles in Goats. *Int J Curr Microbiol App Sci.* 2019;8:2688-2696.
6. Ulutas E, Eryavu A, Bulbul A, Rahman A, Kucukkurt I, Uyarlar C. Effect of Zinc Supplementation on Haematological Parameters, Biochemical Components of Blood and Rumen Fluid, and Accumulation of Zinc in Different Organs of Goats. *Pakistan J. Zool.* 2020;52:977-988.
7. Phiri ECJH, Viva M, Chibunda RT, Mellau ISB. Effect of zinc supplementation on plasma mineral concentration in grazing goats in sub-humid climate of Tanzania. *Tanz Vet J.* 2009;26:92-96.
8. Garg AK, Vishal M, Das RS. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. *Anim Feed Sci Technol.* 2008;144:82-96.
9. Zaboli K, Aliarabi H, Bahari AA, Abbasalipourkabir R. Role of dietary nano-zinc oxide on growth performance and blood levels of mineral: A study on Iranian Angora (Markhoz) goat kids. *JPHCS.* 2013;2:19-26.
10. Mallaki M, Norouzian MA, Khadem AA. Effect of organic zinc supplementation on growth, nutrient utilization, and plasma zinc status in lambs. *Turk J Vet Anim Sci.* 2015;39:75-80.
11. Singh KK, Maity SB, Maity A. Effect of nano zinc oxide on zinc bioavailability and blood biochemical changes in pre-ruminant lambs. *Indian J Anim Sci.* 2018;88:805–807.
12. Belewu A, Adewumi D. Effect of green syntheses nano zinc oxide on performance characteristics and haematobiochemical profile of west African Dwarf goats. *Anim Res Int.* 2021;18:3938–3946.
13. Eryavuz A, Durgan Z, Keskin F. Effects of Ration Supplemented with Zinc on Some Rumen and Blood Parameters, Mohair Production and Quality in Faunated and Defaunated Angora Goats. *Turk J Vet Anim Sci* 2002;26:753-760.

14. Salama AK, Cajat G, Albanell E, Snch X, Casals R. Effects of dietary supplements of zinc-methionine on milk production, udder health and zinc metabolism in dairy goats. *J Dai Sci.* 2003;70:9–17.
15. Jia W, Zhu X, Zhang W, Cheng J, Guo C, Jia Z. Effects of Source of Supplemental Zinc on Performance, Nutrient Digestibility and Plasma Mineral Profile in Cashmere Goats. *Asian-Aust J Anim Sci.* 2008;22:1648 –1653.
16. Nagalakshmi D, Dhanalakshmi K, Himabindu D. Effect of dose and source of supplemental zinc on immune response and oxidative enzymes in lambs. *Vet Res Commun.* 2009;33:631–644.
17. Sethy K, Behera K, Mishra SK, Gupta SK, Sahoo N, Parhi SS, et al. Effect of organic zinc supplementation on growth, metabolic profile and antioxidant status of Ganjam sheep. *Indian J. Anim. Res.* 2018;52:839-842.
18. Bono G, Cairoli F, Tamanini C, Abrate L. Progesterone, estrogen, LH, FSH and PRL concentrations in plasma during the estrous cycle in goat. *Reprod Nutr Develop.* 1983;23:217-222.

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Table 1: Average level of haematological parameters in different nano zinc supplemented groups at different days of observation

Haematological parameters	Treatment	0 th	14 th	28 th	42 th	56 th	70 th	84 th	Day of oestrus
HAEMOGLOBIN (g/dL)	T-0	8.80±0.30	8.53±0.22	8.67±0.15	8.62±0.21	8.63±0.19	8.67±0.23	8.45±0.21	8.68±0.19
	T-1	8.80±0.31	8.82±0.33	8.87±0.46	8.95±0.31	8.68±0.20	9.42±0.53	8.33±0.24	8.77±0.39
	T-2	8.58±0.17	8.50±0.20	9.13±0.41	9.07±0.39	8.95±0.26	8.53±0.25	9.18±0.41	9.45±0.39
	T-3	8.83±0.33	8.67±0.33	8.98±0.25	9.08±0.29	9.18±0.36	8.83±0.23	8.62±0.22	9.72±0.36
PACKED CELL VOLUME (%) Packed cell volume (%)	T-0	25.35±1.96	27.58±0.54	26.03±0.89	24.45±1.39	24.82±0.71	24.68±1.39	26.27±0.57	24.45±1.58
	T-1	27.27±0.98	27.05±0.94	25.6±1.48	22.15±0.72	23.63±0.80	24.63±1.68	25.05±1.72	24.57±1.84
	T-2	25.87±2.35	26.58±2.29	27.7±1.81	26.63±2.15	28.13±1.98	25.38±1.59	26.33±1.34	25.33±2.04
	T-3	25.32±1.67	25.63±2.24	26.88±2.54	25.97±2.22	25.28±1.59	25.85±0.10	27.15±0.69	28.22±1.58
RBC (10 ⁶ /μl)	T-0	15.93±0.17	16.72±0.26	16.87±0.31	16.03±0.53	16.47±0.37	16.30±0.43	16.55±0.35	15.90±0.60
	T-1	16.60±0.40	16.53±0.52	16.65±0.33	16.93±0.42	16.77±0.55	16.47±0.48	17.02±0.25	16.13±0.48
	T-2	16.55±0.49	16.37±0.63	16.17±0.58	15.90±0.21	15.80±0.64	16.25±0.32	16.68±0.24	16.97±0.33

	T-3	15.97±0.69	16.10±0.54	16.60±0.46	16.38±0.25	16.35±0.63	16.20±0.59	16.37±0.34	16.52±0.41
WBC ($10^3/\mu\text{l}$)	T-0	10.05±0.31	9.87±0.36	9.73±0.50	9.78±0.32	10.37±0.36	10.03±0.30	9.77±0.36	9.50±0.49
	T-1	9.97±0.37	9.32±0.20	9.95±0.29	9.72±0.38	9.60±0.20	9.67±0.18	9.45±0.36	10.07±0.29
	T-2	10.08±0.31	9.37±0.14	10.12±0.17	9.57±0.33	9.92±0.18	10.02±0.22	9.48±0.32	9.87±0.17
	T-3	9.78±0.29	9.27±0.30	9.57±0.32	9.33±0.23	9.57±0.21	9.43±0.30	9.25±0.38	9.93±0.31

Table 2: Average level of biochemical parameters in different nano zinc supplemented groups at different days of observation

Biochemical parameters	Treatment	0 th	14 th	28 th	42 th	56 th	70 th	84 th	Day of oestrus
Calcium (mg/dl)	T-0	8.84±0.08	8.98±0.05	8.9±0.03	8.87±0.06	8.83±0.05	8.69±0.14	8.70±0.15	8.67±0.12
	T-1	9.00±0.07	8.90±0.03	8.92±0.07	8.84±0.06	8.89±0.03	8.69±0.08	8.58 ±0.10	8.69±0.03
	T-2	8.93±0.04	8.96±0.06	8.89±0.02	8.86±0.04	8.79±0.03	8.69±0.08	8.84±0.03	8.74±0.04
	T-3	8.89±0.04	8.94±0.04	8.84±0.04	8.78±0.06	8.68±0.05	8.77±0.04	8.70±0.03	8.66±0.02

Phosphorus(mg/dl))	T-0	6.85±0.18	6.50±0.17	6.85±0.17	6.74±0.15	6.58±0.19	6.89±0.16	6.89±0.08	6.77±0.16
	T-1	6.74±0.19	6.68±0.18	6.79±0.16	6.53±0.21	6.52±0.15	6.65±0.11	6.84±0.05	6.62±0.15
	T-2	6.60±0.18	6.72±0.17	6.65±0.18	6.53±0.16	6.46±0.18	6.74±0.12	6.67±0.10	6.59±0.13
	T-3	6.47±0.20	6.64±0.13	6.73±0.17	6.69±0.19	6.61±0.22	6.70±0.21	6.74±0.08	6.72±0.18
Iron (µg/dl)	T-0	56.80±1.05	55.85±0.31	57.18±0.21	56.64±0.23	56.61±0.41	55.81±0.27	55.92±0.46	52.76±0.74
	T-1	56.97±0.79	56.18±0.62	58.62±1.18	56.92±2.61	55.96±1.30	56.35±0.10	57.84±0.49	56.31±0.50
	T-2	57.90±1.37	58.36±1.41	56.61±1.43	55.91±1.92	54.42±1.24	54.92±1.35	55.31±0.96	54.04±0.94
	T-3	57.63±0.34	58.02±0.42	56.65±0.72	56.30±0.52	56.09±0.38	56.45±0.67	57.92±0.54	55.00±0.56
Zinc (µg/dl)	T-0	0.72±0.03 ^a	0.75±0.03 ^a	0.78±0.02 ^a	0.73±0.03 ^a	0.80±0.01 ^a	0.74±0.02 ^a	0.77±0.02 ^a	0.75±0.02 ^a
	T-1	0.92±0.03 ^b	0.95±0.02 ^b	0.95±0.03 ^b	1.02±0.02 ^b	1.00±0.03 ^b	1.02±0.04 ^b	1.01±0.04 ^b	1.04±0.02 ^b
	T-2	0.89±0.02 ^b	0.97±0.02 ^b	0.95±0.02 ^b	0.90±0.02 ^c	1.00±0.03 ^b	1.05±0.04 ^b	1.10±0.03 ^b	1.14±0.03 ^b
	T-3	0.89±0.04 ^b	1.06±0.04 ^c	1.25±0.03 ^c	1.20±0.03 ^d	1.31±0.02 ^c	1.33±0.02 ^c	1.41±0.02 ^c	1.45±0.02 ^c
Catalase activity (U/mg protein)	T-0	1.29±0.01 ^a	1.3±0.01 ^a	1.30±0.01 ^a	1.31±0.01 ^a	1.32±0.01 ^a	1.31±0.01 ^a	1.31±0.01 ^a	1.29±0.01 ^a
	T-1	1.37±0.02 ^b	1.92±0.03 ^b	2.09±0.02 ^b	2.24±0.02 ^b	2.27±0.02 ^b	2.30±0.01 ^b	2.32±0.01 ^b	2.22±0.02 ^b
	T-2	1.38±0.01 ^b	1.89±0.03 ^b	2.04±0.03 ^b	2.18±0.03 ^b	2.06±0.16 ^b	2.27±0.01 ^b	2.28±0.01 ^b	2.16±0.02 ^b

	T-3	1.29±0.004 ^a	1.30±0.01 ^a	1.31±0.01 ^a	1.33±0.01 ^a	1.36±0.01 ^a	1.37±0.01 ^c	1.38±0.01 ^c	1.28±0.01 ^a
Superoxide dismutase (SOD) activity (U/mg protein)	T-0	19.88±0.29 ^a	20.35±0.26 ^a	20.20±0.19 ^a	21.17±0.17 ^a	21.41±0.07 ^a	21.32±0.09 ^a	21.36±0.06 ^a	19.92±0.14 ^a
	T-1	19.61±0.16 ^a	20.45±0.32 ^a	22.76±0.40 ^b	24.90±0.30 ^b	26.63±0.39 ^b	28.37±0.32 ^b	29.10±0.26 ^b	23.7±0.44 ^b
	T-2	19.70±0.19 ^a	20.61±0.33 ^a	23.92±0.36 ^c	26.94±0.31 ^c	28.93±0.14 ^c	29.68±0.22 ^c	30.17±0.14 ^c	23.90±0.34 ^b
	T-3	19.9±0.27 ^a	20.38±0.25 ^a	20.38±0.15 ^a	21.32±0.19 ^a	21.86±0.19 ^a	22.2±0.11 ^d	22.44±0.09 ^d	19.52±0.12 ^a
Estrogen (pg/ml)	T-0	61.13±3.08	59.25±2.94	56.9±2.78	55.81±2.64	55.19±2.83	53.91±2.55	52.47±2.52	79.57±3.50
	T-1	59.75±2.3	58.16±2.62	55.47±2.87	54.31±3.11	53.84±2.63	50.71±3.27	49.67±3.18	80.87±2.14
	T-2	63.2±2.17	60.7±2.44	58.16±2.12	56.8±2.01	55.39±2.22	54.17±2.18	40.72±5.13	76.29±5.74
	T-3	60.59±2.43	59.18±2.75	58.56±2.67	57.18±2.47	55.95±2.6	49.98±4.25	47.92±4.01	83.95±3.22
Progesterone (ng/ml)	T-0	3.3±0.21 ^a	3.27±0.26 ^a	3.59±0.13 ^a	3.74±0.2 ^a	3.53±0.14 ^a	3.42±0.12 ^a	3.05±0.07 ^a	0.74±0.20 ^a
	T-1	3.02±0.24 ^a	3.29±0.19 ^a	3.38±0.15 ^a	3.51±0.13 ^a	3.61±0.13 ^a	3.65±0.14 ^a	3.32±0.23 ^a	0.83±0.16 ^a
	T-2	3.44±0.25 ^a	3.35±0.16 ^a	3.61±0.14 ^a	3.5±0.23 ^a	3.51±0.18 ^a	2.88±0.22 ^a	4.66±0.46 ^b	1.07±0.05 ^a
	T-3	3.41±0.44 ^a	3.08±0.25 ^a	3.33±0.2 ^a	3.04±0.24 ^a	2.4±0.35 ^b	5.25±0.47 ^b	5.69±1.04 ^c	0.77±0.18 ^a