

Research Article



Effects of Rumen-Protected D-Aspartate and Zinc Bio-Complex on Performance of Growing Male Goats Fed High-Concentrate Diets

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Abstract | This study aimed to evaluate the effects of rumen-protected D-aspartate and zinc bio-complex on feed intake, nutrient intake, blood profiles, blood metabolites, and hormone concentration of growing male goats. Eight cross-bred Boer (BW = 22.6 ± 0.62 kg) growing male goats at approximately 12 months of age were assigned in a replicated 4x4 Latin square design. Treatments were as follows: 1) control with no supplementation (CON), 2) rumen-protected D-Aspartate (RDA), at 132.74 mg/kg body weight (BW), 3) zinc bio-complex (ZBC) at 73.74 mg/kg BW, and 4) supplementation of RDA at 132.74 mg/kg BW plus ZBC at 73.74 mg/kg BW (RAZC). All dietary treatments were provided in gelatin hard capsules prepared for each treatment except the control. All animals were individually fed roughage to concentrate at a ratio of 30 to 70 daily. The experiment consisted of four consecutive periods, with the first 14 d for dietary adaptation and the last 6 d for data collection each, and lasted for 80 days of overall trial. Results indicated that all supplementations did not affect feed and nutrient intake ($p > 0.05$). However, adding RDA, ZBC, and RAZC significantly increased blood hematocrit ($p = 0.010$). Adding either RDA or ZBC and RAZC combination did not affect blood metabolites regarding glucose ($p = 0.074$) or blood urea nitrogen ($p = 0.364$). Creatine was the lowest in goats fed RDA but the greatest in ZBC ($p = 0.003$) compared with CON and RAZC. Supplementation of RDA, ZBC, and RAZC affected concentrations of free T4 hormones, especially for ZBC ($p = 0.008$). However, there were no effects on free T3 ($p = 0.738$), TSH ($p = 0.403$), and testosterone ($p = 0.671$). These results suggest that supplements did not significantly affect feed and nutrient intake, complete blood count, blood metabolites, and hormones. However, there was little benefit to animal health when growing male goats were fed high-concentrate diets. Further longer-term trials should be conducted to determine additional effects on productive performance and changes in semen quality.

Keywords | Rumen-protected D-aspartate, Zinc Bio-complex, Growing male goat

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INTRODUCTION

Goats are important small ruminants and quite popular among smallholder farmers with limited land hold-

ings due to their small body size; they require less space for raising, can consume both tree leaves and grasses, but efficiently produce and yield products more rapidly than cattle in terms of meat, milk, leather, and fur. Maintaining ap-

appropriate numbers of male goats in herds is crucial because they contribute half of the genetic makeup of newborn kids and reflect the characteristics of future herds (Bearden and Fuquay, 2000; Kumar et al., 2023). During the limit of high-quality roughages, feeding goats using a high ratio of concentrate diets is a common regime adopted by smallholder farmers because of their availability in local markets, high nutritive values, high digestibility, and proficient maintain herd productive efficiency (Yousuf et al., 2014; Phreyamittrachaiya and Wittayakun, 2021; Wang et al., 2023).

D-aspartate (D-Asp) is a non-essential amino acid that can be synthesized and found in various animal tissues and plays crucial roles in the biosynthesis and secretion of hormones in endocrine systems (Roshanzamir and Safavi, 2017; Li et al., 2018). Effects of D-Asp in enhancing semen quality and increasing brain sex hormones have been described for non-ruminants (Ansari et al., 2017; Fioer et al., 2018). A previous study by Ahmad and Atiyah (2022) demonstrated significant effects on some biochemical blood traits and the level of pituitary sex hormones after D-Asp injection into adult male goats. At the same time, Fayhan and Ahmad (2022) reported significant improvement in the semen quality of adult male goats after injecting D-Asp. However, minimal work has been carried out to examine D-Asp effects in the form of rumen-protected amino acids on productive performance, especially in growing male goats.

Zinc is an element involved in various functions in the animal body. It affects animal growth, reproductive function, bone and blood formation, metabolism of nucleic acids, proteins, and carbohydrates, an activator of several enzyme systems, and secretion of hormones (McDowell, 1992; McDonald et al., 2002). Dietary zinc supplementation enhances the productive performance of small ruminants, depending on the production level, source, and dosage of zinc (Angeles-Hernandez et al., 2021), while deficiency results in skin lesions of both hyperkeratosis and parakeratosis, abnormal hoof growth, anorexia, and depression (Nelson et al., 1984; Krametter-Froetscher et al., 2005). Because zinc absorption is low in efficiency in small animal intestines, combining these minerals with some amino acids as chelating agents increases its absorption in the guts and bioavailability (McDowell, 1992; Jacob et al., 2022). Moreover, zinc in organic and inorganic complexes enhances some productive performances, metabolism, and physiological responses when supplemented in goat diets (Pechova et al., 2009; Abu El-Ella et al., 2014; Soliman and Min, 2019).

Nevertheless, to our knowledge, the effects of rumen-protected D-Asp and zinc in the forms of bio-complex, adding to the diets of growing male goats, are still scarce. We

hypothesized that better productive and physiological responses would be observed in growing male goats receiving rumen-protected D-Asp or zinc bio-complex. Therefore, the present study was conducted to examine the effects of adding rumen-protected D-Asp, zinc bio-complex, and a combination of both on feed intake, nutrient intake, blood profiles, blood metabolites, and hormone concentration of crossbred Boer growing male goats under high-concentrate feeding conditions.

MATERIALS AND METHODS

The materials and methods used in this experiment were approved by the Rajamangala University of Technology Animal Care and Use Committee, license no. U1-06718-2560. Animal care and procedures were performed according to the Animal for Thai Scientific Purposes Acts guidelines, B.E. 2558 or A.D. 2015 (TGG, 2015).

ANIMALS AND EXPERIMENTAL DESIGN

Eight crossbred Boer-growing male kids, an average of 12 months of age and 22.6 kg body weight, were assigned in two replicated 4x4 Latin square designs. The four dietary treatments were as follows: 1) control with no supplementation (CON), 2) rumen-protected D-Aspartate (Activelab®, Poland), at 132.74 mg/kg BW (RDA), 3) zinc bio-complex (Blackmores®, Australia) at 73.74 mg/kg BW (ZBC), and 4) supplementation of RDA at 132.74 mg/kg BW plus ZBC at 73.74 mg/kg BW (RAZC). All dietary treatments were provided in gelatin hard capsules prepared for each treatment except the control. Besides, Pangola grass hay (PGH) and commercial concentrate (CC) at a ratio of 30 to 70 were fed twice daily at 07.00 a.m. and 04.00 p.m. Each animal was raised individually in a 1.5 x 2.0 m² pen with free access to roughage, water, and mineral blocks. The experimental periods consisted of four consecutive periods and lasted 80 days of the trial. Each period consisted of a 14-day adapt period and six days for sampling and measurements. PGH was purchased from a local community enterprise group that cultivated grass for sale at Ban Tha Som Poi, Lampang, Thailand. The feeding trial was conducted at a farm unit at the Rajamangala University of Technology Lanna, Lampang campus, Lampang province (N18°17'32.35", E 99°29'33.97"), northern Thailand.

SAMPLING AND DATA COLLECTION

The amounts of offered feed and refusal were recorded daily. In the first seven days of each adaptation period, feed ingredients were collected and dried at 60°C in a hot air oven for 72 h for DM daily feed intake adjustment. All goats were weighed twice (day 2 and day 20) during each period. Feed samples were collected, dried at 60°C for 72 h, ground, and composited to analyze for dry matter (DM),

crude protein (CP), ether extract (EE), crude fiber (CF), and ash (AOAC, 1990). Nitrogen-free extract (NFE) was calculated as follows: $NFE = \% DM - (\% EE + \% CP + \% Ash + \% CF)$ (Van Soest, 1982). Neutral detergent fiber (NDF) was measured using the method of Van Soest et al. (1991). Organic matter (OM) was calculated as follows: $OM = 100 - Ash \%$. During the last 3-d of each data collection period, fecal grab samples were collected twice daily at 12 h intervals, pooled on an equal wet-weight basis for each goat, dried at 60°C for 72 h, ground, and analyzed for DM by the method of AOAC (1990), and AIA by the method of Van Keulen and Young (1977). Digestibility coefficients of nutrients were calculated in equations given by Schneider and Flatt (1975) as follows: $DM \text{ digestibility, } \% = 100 - [100 \times (AIA\% \text{ in feed}) \div (AIA \% \text{ in feces})]$. On the last day of each experimental period, approximately 10 ml of blood samples were taken from the jugular vein at 4 h after feeding and subsequent analysis for complete blood count using Automated Hematology Analyzer (Model URIT-3000 Plus, URIT Medical Electronic Co, Ltd., China); biochemical profiles using Auto Biochemistry Analyzer (Model TC 220, Tecom Science Corporation, China); free triiodothyronine (FT3) and free thyroxine (FT4), thyroid stimulating hormone (TSH) using immunoassay analyzer (ARCHITECT i2000SR PLUS, Abbott, USA) and testosterone using immunoassay analyzer (Model DXI800, Beckman Coulter, Inc., USA).

STATISTICAL ANALYSIS

Data from both replications were averaged and then analyzed using a general linear procedure for Analysis of Variance (ANOVA), and significance was set at a *p*-value less than 0.05. Treatment means were compared by least significant difference (SPSS, 2019). The statistical model used was described by: $Y_{ijkl} = \mu + goat_{i(i)} + treatment_j + period_k + square_l + \epsilon_{ijkl}$ where Y_{ijkl} is the dependent variable, μ is the overall mean, $goat_{i(i)}$ is the goat effect inside each square (*i* = 1, 2, 3, 4, 5, 6, 7, or 8), $treatment_j$ is the effect of treatments (*j* = 1, 2, 3, or 4), $period_k$ is the effect of each period (*k* = 1, 2, 3, or 4), $square_l$ is the effect of square (*l* = 1 or 2), and ϵ_{ijkl} is the experimental error. Differences were declared significant at *p* < 0.05.

RESULTS AND DISCUSSION

NUTRIENT COMPOSITION OF DIETS

Nutrient compositions of PG, and CC fed to growing male goats are shown in Table 1. The quality of PGH was relatively poor according to its chemical composition, which was low in energy (TDN) and crude protein (CP), but high in neutral detergent fiber (NDF). In contrast, CC had a higher quality regarding CP, NDF, and TDN contents than PGH.

Table 1: Nutrient contents of experimental diets, % DM.

Items	CC	PGH
DM	97.53	95.47
OM	89.02	92.70
CP	21.14	6.62
EE	5.35	1.74
NDF	35.17	71.18
ADF	20.84	44.42
CF	15.47	35.00
Ash	10.98	7.30
NFE ¹	44.59	44.81
TDN ²	81.74	57.72

Note: CC: commercial concentrate. PGH: Pangola grass hay. DM: Dry matter. OM: Organic matter. CP: Crude protein. EE: Ether extract. NDF: Neutral detergent fiber. ADF: Acid detergent fiber. CF: Crude fiber. NFE: ¹Nitrogen-free extract = $\% DM - (\% EE + \% CP + \% Ash + \% CF)$ (Van Soest, 1982). ²TDN: Total digestible nutrient = $105.2 - (0.667 \times \% NDF)$ (Undersander et al., 1993).

FEED INTAKE AND NUTRIENT INTAKE

Feed intake, nutrient intake, and apparent digestibility are presented in Table 2. Inclusions of RDA, ZBC, and RAZC in diets had no significant effects on daily dry matter intake of grass (*p* = 0.942), concentrate (*p* = 0.090), and total feed intake (*p* = 0.280). Daily nutrient intake from grass, concentrate, and total feed intake as expressed in terms of organic matter, crude protein, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were unaffected by supplementation of RDA, ZBC, and RAZC in diets (*p* > 0.05). The findings from this study suggest that supplementing either RDA, ZC, or DABZ has no effects on feed intake in terms of dietary roughage, concentrate, and total feed. In non-ruminants, low dosages of D-aspartate supplementation have been reported to benefit both improvements in growth performance and stress resistance (Li et al., 2018), but decreased feed intake in high dosages (Erwan et al., 2013).

Based on zinc chelate supplementation, the results are in agreement with those previous trials that reported zinc or zinc amino acid complex supplement did not affect the intake of roughage (Chaudhary et al., 2016; Soliaman and Min, 2019), concentrate (Soliaman and Min, 2019), and total feed intake (Garg et al., 2008; Aditia et al., 2014; Chaudhary et al., 2016; Soliaman and Min, 2019) in growing goats. Because zinc is a trace element that mainly involves and functions in subcellular metabolism, its impact may not be measured by animal feed intake. The apparent digestibility of total diets was similar and ranged from 55.88 to 64.03 % of DM (*p* = 0.297). These findings agreed with previous work on Muzaffarnagar male lambs by Garg et al. (2008). The apparent digestibility of total diets is rel

Table 2: Feed and nutrient intake.

Items	Control	RDA	ZBC	RAZC	SE	p-value
Roughage intake						
DM						
- %BW	0.62	0.56	0.53	0.58	0.20	0.938
- g/d	126.02	113.90	122.67	129.20	37.34	0.942
OM, g/d	121.39	109.86	118.09	125.34	36.33	0.983
CP, g/d	8.69	7.79	8.47	8.82	2.67	0.946
NDF, g/d	91.65	84.43	90.60	96.54	28.84	0.946
ADF, g/d	58.02	52.44	56.45	59.95	17.56	0.938
Concentrate intake						
DM						
-%BW	1.07	1.18	1.14	1.48	0.20	0.102
- g/d	238.84	264.72	244.11	322.72	41.06	0.090
OM, g/d	217.05	240.72	221.68	293.57	37.37	0.089
CP, g/d	51.60	56.98	52.28	69.98	9.19	0.092
NDF, g/d	86.62	96.15	88.67	116.22	14.41	0.089
ADF, g/d	51.09	56.90	52.48	52.44	19.66	0.976
Total feed intake						
DM						
-% BW	1.69	1.74	1.90	2.06	0.36	0.501
- g/d	364.86	378.62	366.78	451.93	64.91	0.280
OM, g/d	338.44	350.59	339.78	418.91	60.67	0.285
CP, g/d	60.30	64.77	60.76	78.81	10.46	0.135
NDF, g/d	178.27	180.47	179.27	212.79	36.83	0.525
ADF, g/d	109.12	109.27	108.93	128.88	28.35	0.536
Apparent digestibility of total diets						
DM, %	59.21	62.53	55.88	64.03	5.85	0.297
Average body weight, kg	23.11	21.70	22.70	22.89	1.05	0.333

Note: Control: No supplementation (CON). RDA: Rumen-protected D-Aspartate at 132.74 mg/kgBW. ZBC: Zinc bio-complex at 73.74 mg/kgBW. RAZC: RDA at 132.74 mg/kgBW + ZBC at 73.74 mg/kgBW. BW: Bodyweight. DM: Dry matter. OM: Organic matter. CP: Crude protein. NDF: Neutral detergent fiber. ADF: Acid detergent fiber.

Table 3: Complete blood count.

Items	Control	RDA	ZBC	RAZC	SE	p-value
Hct, mg/dL	20.88 ^a	24.25 ^b	27.25 ^c	24.50 ^b	1.66	0.010
Hb, mg/dL	7.91	7.16	7.65	7.90	0.60	0.345
WBC, 10 ³ million/cu.m.m	14.88	15.87	17.08	15.47	1.08	0.122
Platelet, 10 ⁶ million/cu.m.m	2.26	2.13	1.76	2.15	0.34	0.286
PMN, 10 ⁶ million/cu.m.m	59.40	56.87	56.75	60.25	5.69	0.766
Lymph, %	36.37	37.50	37.12	35.87	4.66	0.958
Eosinophil, %	2.87	3.87	4.50	2.25	1.70	0.335
Monocyte, %	1.25	1.75	1.62	1.62	0.54	0.615

Note: Control: No supplementation (CON). RDA: Rumen-protected D-Aspartate at 132.74 mg/kgBW. ZBC: Zinc bio-complex at 73.74 mg/kgBW. RAZC: RDA at 132.74 mg/kgBW + ZBC at 73.74 mg/kgBW. BW: Bodyweight. Hb: Hemoglobin. Hct: Hematocrit. WBC: White blood cell count. PMN: Polymorphonuclear neutrophils.

actively low due to high NDF and ADF in PGH. Wang et al. (2023) reported higher apparent digestibility of low, medium, and high concentrate diets, ranging from 62.70 to 79.44 % of DM due to temperate roughages, i.e., oat grass

Table 4: Blood metabolites and hormone concentrations.

Items	Control	RDA	ZBC	RAZC	SE	p-value
Glucose, mg/dL	72.27	74.50	72.87	69.25	7.13	0.074
BUN, mg/dL	20.62	20.37	22.12	24.25	3.15	0.364
Creatine, mg/dL	0.68 ^a	0.62 ^b	0.77 ^c	0.64 ^a	0.03	0.003
Hormones						
TSH, uIU/mL	0.02	0.02	0.01	0.01	0.01	0.403
Free T4, ng/dL	0.86 ^a	0.99 ^b	0.76 ^c	0.92 ^{ab}	0.06	0.008
Free T3, pg/mL	2.98	3.12	3.35	3.32	0.52	0.738
Testosterone, ng/mL	4.22	5.34	3.15	3.56	2.59	0.671

Note: Control: No supplementation (CON). RDA: Rumen-protected D-Aspartate at 132.74 mg/kgBW. ZBC: Zinc bio-complex at 73.74 mg/kgBW. RAZC: RDA at 132.74 mg/kgBW + ZBC at 73.74 mg/kgBW. BW: Bodyweight. BUN: Blood urea nitrogen. Free T3: Triiodothyronine. Free T4: Thyroxine. TSH: Thyroid stimulating hormone.

and wheat straw were being used as feed ingredients.

COMPLETE BLOOD COUNT

Complete blood count is shown in Table 3. Complete blood count is generally an indicator of monitoring health and a wide range of body conditions. In this trial, supplementation of RDA, ZBC, and RAZC did not have significant effects on complete blood counts as expressed in hemoglobin (Hb) ($p = 0.345$) and white blood cell count (WBC) ($p = 0.122$). All groups had low hemoglobin levels, ranging from 7.16 to 7.91 mg/dL. The reference average hemoglobin values in goats range from 8.0 to 12.0 g/dL (Blood and Studdert, 1995; Jackson and Cockcroft, 2002). However, ZC supplementation had significantly affected blood hematocrit (Hct) ($p = 0.010$), higher than both RDA and RAZC; that means the ZBC supplementation benefits growing goat kids that may increase the size, volume, number, or functional capacity of erythrocytes in blood. This agrees with Adam et al. (2015) and Yuherman et al. (2017), who reported a positive correlation between diet zinc levels and blood hematocrit. Adequate concentrations of zinc in the serum and the diets are vital and beneficial in increasing the functions and efficiency of the reproductive system (Goncalves et al., 2014). RDA and RAZC combination had similar effects ($p > 0.05$) on blood hematocrit but were significantly higher when compared to the control group ($p = 0.010$). Adding RDA, ZBC, and RAZC did not significantly affect platelet concentration ($p = 0.286$), polymorphonuclear neutrophil (PMN) ($p = 0.766$), lymphocyte ($p = 0.958$), eosinophil ($p = 0.335$), and monocyte ($p = 0.615$); imply that RDA, ZBC, and RAZC may not influence animal immune systems.

BLOOD METABOLITES AND HORMONE CONCENTRATIONS

Blood metabolites and hormone concentrations are shown in Table 4. All supplements did not affect blood glucose ($p = 0.074$). The concentration of plasma glucose for the control diet was 72.27 mg/dL, whereas goats that were

provided RDA, ZBC, and RAZC averaged 74.50, 72.87, and 69.25 mg/dL, respectively; the means of plasma glucose in all groups were within the reference values for goats from about 50 to 75 mg/dL (Kaneko et al., 2008). In this study, plasma glucose was relatively high due to feeding the higher ratio of CC to PGH and higher consumption of CC in all groups, which enhance propionic acid production in the rumen, which later on undergoes gluconeogenesis by the liver, resulting in high plasma glucose (Wang et al., 2023). Blood urea nitrogen (BUN) was not different among goats offered RDA, ZBC, or RAZC ($p = 0.364$). RDA and RAZC were contained in the gelatin capsule, which may escape fermentation in reticulo-rumen or low proteolytic activity in the ruminal microflora. Pena et al. (2023) reported that gelatin capsules could reduce their degradation rate in rumen. Generally, the reference value of BUN for goats ranges from 15 to 33 mg/dL (Blood and Studdert, 1995) or 12 to 26 mg/dL (Jackson and Cockcroft, 2002). Adding RDA, ZBC, and RAZC affected creatine, with the highest value in ZBC, followed by RAZC and RDA, respectively ($p = 0.003$). Generally, serum creatinine level is a valuable indicator of glomerular filtration in the kidney (Ranasinghe et al., 2015). This suggested that RDA enhanced kidney function efficiency due to the lowest creatine at 0.62 mg/dL, followed by RAZC at 0.64 mg/dL, while ZBC tended to load kidney function with the highest creatine at 0.77 mg/dL. However, the reference range of serum creatine in goats is 0.6 to 1.6 mg/dL (Jackson and Cockcroft, 2002). Dietary supplementation of RDA, ZBC, and RAZC did not affect thyroid-stimulating hormone (TSH) levels ($p = 0.403$). TSH, produced by the pituitary gland, is being used to monitor the thyroid gland; it functions by binding to TSH receptors in thyroid gland to stimulate the release of thyroxine (T4) and a small amount of tri-iodothyronine (T3), which are mostly bound to plasma proteins, but some remain in free forms as free T4 and T3 (Todini, 2007). Circulating thyroid hormones reflect the energy balance and nutritional and metabolic status crucial to sustaining domestic animals' productive

performance (Todini, 2007; Aghwan et al., 2013). In our study, adding RDA influenced higher free T4 concentration ($p = 0.008$); in contrast, ZBC influenced lower free T4 concentration ($p = 0.008$). However, free T4 values obtained from the goats included in the present study were in the normal range by published physiological ranges reported in adult nonpregnant goats by Todini (2007), ranging from 0.51 to 1.65 ng/dL; McDonald et al. (1988) and Celi et al. (2008) ranged from 0.51 to 1.71 ng/dL. RDA, ZBC, and RAZC supplementation had no effect on testosterone concentration ($p = 0.671$).

CONCLUSION

The current experiment evaluated the influence of manipulating the supplementary RDA and ZBC to diets containing a high ratio of concentrate to roughage on feed intake, complete blood count, blood metabolites, and hormones. Our results suggest that supplements did not significantly affect feed and nutrient intake, complete blood count, blood metabolites, and hormones. However, there was little benefit of supplements to animal health when growing male goats were fed high-concentrate diets.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

NOVELTY STATEMENT

This study has explored the effects of rumen-protected D-aspartate and zinc bio-complex on the productive performance of growing male goats. Our results demonstrated that the parameters for improving feed intake, nutrient intake, blood metabolites, and hormone concentration were not identified. However, adding rumen-protected D-aspartate potentially promotes animal health and renal function in terms of hematocrit and creatine compared to other groups when fed high-concentrate diets.

AUTHORS CONTRIBUTION

All authors contributed equally according to their tasks

and approved the final manuscript.

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