

Effects of Different Nutritional Regulation Methods on Growth and Development, Hematological Parameters, Plasma Antioxidant Capacity, and Immune Indices in Stunted Yaks (Postprint)

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Abstract

This study aimed to identify methods for alleviating oxidative and immune stress and promoting growth in stunted yaks through nutritional regulation. Forty 1-year-old Qinghai plateau-type stunted yaks and 10 normal yaks were selected based on the criterion of being less than 1.5 standard deviations below the mean body weight of their same-age, same-breed cohort. The 40 stunted yaks were randomly allocated to 4 groups based on body weight: a stunted grazing group, a concentrate supplementation group, a cysteamine group (supplemented with concentrate plus 80 mg/kg BW cysteamine), and a yeast group (supplemented with concentrate plus 0.3% active dry yeast). Normal yaks were designated as the normal grazing group, with 5 replicates per group and 2 yaks per replicate. The preliminary period was 15 days, and the experimental period was 60 days. The results showed: 1) Compared with the stunted grazing group, the concentrate supplementation, cysteamine, and yeast groups significantly improved the body measurement indices of stunted yaks ($P < 0.05$). 2) The concentrate supplementation group had significantly higher blood erythrocyte count and hemoglobin content than the stunted grazing group ($P < 0.05$), and significantly lower blood leukocyte count than the stunted grazing group ($P < 0.05$). 3) On day 1, the normal grazing group had significantly lower plasma malondialdehyde (MDA) content and significantly higher total superoxide dismutase (T-SOD) activity, glutathione peroxidase (GSH-Px) activity, and total antioxidant capacity (T-AOC) than the other 4 groups ($P < 0.05$). On day 60, the cysteamine and yeast groups of stunted yaks had significantly lower plasma MDA content than day 1 ($P < 0.05$), and significantly lower than the stunted grazing group ($P < 0.05$); plasma T-SOD activity and T-AOC were significantly higher than day 1 ($P < 0.05$), and significantly higher than the stunted grazing

group ($P < 0.05$); the yeast group had significantly higher plasma GSH-Px activity than day 1 ($P < 0.05$), and significantly higher than the stunted grazing group ($P < 0.05$). 4) On day 1, the normal grazing group had significantly higher plasma immunoglobulin A (IgA) and immunoglobulin G (IgG) contents than the other 4 groups ($P < 0.05$). On day 60, the concentrate supplementation group had significantly higher plasma IgG content than day 1 ($P < 0.05$), and significantly higher than the stunted grazing group ($P < 0.05$); the cysteamine group had significantly higher plasma IgA content than day 1 ($P < 0.05$), and significantly higher than the stunted grazing group ($P < 0.05$); the yeast group had significantly higher plasma IgA and IgG contents than day 1 ($P < 0.05$), and significantly higher than the stunted grazing group ($P < 0.05$). The results suggest that concentrate supplementation and the addition of 80 mg/kg BW cysteamine or 0.3% active dry yeast to concentrate can improve the antioxidant and immune capacity of stunted yaks and promote compensatory growth, with concentrate supplementation plus 0.3% active dry yeast showing the best effect.

Full Text

Effects of Different Nutritional Regulations on Growth and Development, Blood Routine, and Plasma Antioxidant and Immune Indexes of Yaks with Growth Retardation

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Abstract

This study aimed to identify nutritional regulation strategies to alleviate oxidative and immune stress while promoting growth in yaks with growth retardation. Forty 1-year-old Qinghai high-plateau yaks with growth retardation (selected based on being 1.5 standard deviations below the average weight of same-age, same-breed yak populations) were randomly assigned to four groups: farming yaks with growth retardation group, concentrate supplementation group, cysteamine group (concentrate + 80 mg/kg BW cysteamine), and yeast group (concentrate + 0.3% active dry yeast). An additional ten 1-year-old Qinghai high-plateau yaks with normal growth performance served as the farming healthy yaks group. Each group comprised 5 replicates with 2 yaks per replicate. The pre-trial period lasted 15 days, followed by a 60-day formal trial period.

The results demonstrated: (1) Compared with the farming yaks with growth

retardation group, the concentrate, cysteamine, and yeast groups showed significant improvements in body measurements ($P < 0.05$). (2) The concentrate group exhibited significantly higher red blood cell count and hemoglobin content, along with significantly lower white blood cell count, compared to the farming yaks with growth retardation group ($P < 0.05$). (3) On day 1, the farming healthy yaks group showed significantly lower plasma malondialdehyde (MDA) content and significantly higher plasma total superoxide dismutase (T-SOD) activity, glutathione peroxidase (GSH-Px) activity, and total antioxidant capacity (T-AOC) compared to the other four groups ($P < 0.05$). On day 60, the cysteamine and yeast groups displayed significantly reduced plasma MDA content compared to day 1 ($P < 0.05$), which was also significantly lower than that of the farming yaks with growth retardation group ($P < 0.05$). These groups also showed significantly increased plasma T-SOD activity and T-AOC compared to day 1 ($P < 0.05$), significantly exceeding the levels in the farming yaks with growth retardation group ($P < 0.05$). The yeast group additionally demonstrated significantly higher plasma GSH-Px activity compared to day 1 ($P < 0.05$), significantly surpassing the farming yaks with growth retardation group ($P < 0.05$). (4) On day 1, the farming healthy yaks group had significantly higher plasma immunoglobulin A (IgA) and immunoglobulin G (IgG) contents than the other four groups ($P < 0.05$). On day 60, the concentrate group showed significantly higher plasma IgG content compared to day 1 ($P < 0.05$), significantly exceeding the farming yaks with growth retardation group ($P < 0.05$). The cysteamine group exhibited significantly higher plasma IgA content compared to day 1 ($P < 0.05$), significantly higher than the farming yaks with growth retardation group ($P < 0.05$). The yeast group demonstrated significantly increased plasma IgA and IgG contents compared to day 1 ($P < 0.05$), significantly surpassing the farming yaks with growth retardation group ($P < 0.05$).

These findings indicate that concentrate supplementation, particularly with the addition of 80 mg/kg BW cysteamine or 0.3% active dry yeast, can enhance antioxidant capacity and immunity while promoting compensatory growth in yaks with growth retardation. The combination of concentrate supplementation with 0.3% active dry yeast yielded the most effective results.

Keywords: yak with growth retardation; body measurement; antioxidant; immune; cysteamine; active dry yeast

Introduction

Yaks are traditional and dominant livestock on the Qinghai-Tibet Plateau pastoral areas, serving as essential livelihood resources for herders and forming a pillar industry in Tibetan regions. However, under traditional grazing production systems that depend entirely on natural conditions, yak nutrient intake is affected by seasonal variations in forage yield and nutritional quality. When

forage supply is insufficient or nutritional levels are too low, pregnant cows and newborn calves experience chronic nutritional deficiencies, leading to retarded or even stalled postnatal growth and development, ultimately resulting in “stunted yaks” [1]. Stunted yaks exhibit poor feed conversion, small and emaciated body size, high morbidity and mortality rates, and growth performance far below that of same-age, same-breed cattle, thereby reducing the economic benefits of yak farming for herders.

Several primary factors contribute to the formation of stunted yaks. First, the traditional grazing production system in pastoral areas, combined with harsh plateau environments and long cold seasons, causes sharp declines in forage supply and nutritional levels [2]. This results in pregnant yaks experiencing chronic undernutrition, ultimately causing intrauterine growth retardation and impeding postnatal growth and development [3]. Second, due to insufficient nutrient intake, nursing yaks cannot maintain adequate milk yield and quality. Combined with herders’ demand for yak milk, this creates competition between humans and calves for milk, leading to inadequate nutrient intake for calves and retarded growth [4]. Third, the harsh plateau pastoral environment results in high disease incidence in yak calves, and subhealthy calves are prone to growth retardation [5]. Our previous research indicated that insufficient secretion of growth axis hormones is a primary cause of growth retardation in stunted yaks, and that adding cysteamine to concentrate can promote growth axis hormone secretion, thereby stimulating growth and development [6]. Studies have found that oxidative stress in mammalian cells can damage cellular macromolecules and cause organ dysfunction, reducing production performance and even causing various diseases [7]. Meanwhile, immune stress can also alter livestock behavior, metabolism, and neurosecretion, ultimately inhibiting growth. Therefore, subhealthy stunted yaks may be affected by both oxidative and immune stress, though no relevant reports currently exist. Research has shown that cysteamine and active dry yeast can improve animal antioxidant and immune capacity [8-9], and that concentrate supplementation for stunted yaks, as well as adding cysteamine to concentrate, can promote body measurement and gastrointestinal development [1].

Given these considerations, this study investigated the effects of different nutritional regulation methods on growth performance, blood routine, and plasma antioxidant and immune indexes in stunted yaks. The objective was to explore the potential for nutritional interventions to initiate compensatory growth effects in stunted yaks, providing technical support for alleviating the problem of stunted yaks in pastoral areas and promoting efficient and healthy yak production.

Materials and Methods

1.1 Experimental Animals

Based on the criterion of being 1.5 standard deviations below the average weight of same-age, same-breed yak populations [10], forty 1-year-old Qinghai high-plateau yaks with growth retardation were selected from natural grazing conditions, with an average body weight of (72.7 ± 6.0) kg. Additionally, ten 1-year-old Qinghai high-plateau yaks with normal growth performance were selected under the same conditions, with an average body weight of (93.5 ± 6.0) kg.

1.2 Experimental Design

The 40 stunted yaks were randomly assigned to four groups based on body weight: farming yaks with growth retardation group, concentrate group (concentrate supplementation), cysteamine group (concentrate + 80 mg/kg BW cysteamine), and yeast group (concentrate + 0.3% active dry yeast). Normal yaks served as the farming healthy yaks group. Each group consisted of 5 replicates with 2 yaks per replicate. The pre-trial period lasted 15 days (during which the farming yaks with growth retardation group and farming healthy yaks group were grazed, while the other three groups were housed and fed the same basal diet), followed by a 60-day formal trial period.

Farming yaks with growth retardation group: Natural grazing without concentrate supplementation.

Concentrate group: Housed and fed basal diet under barn conditions.

Cysteamine group: Housed and fed basal diet with cysteamine (effective content 30%, Shanghai Xumu Lian Biotechnology Co., Ltd.) added to concentrate at 80 mg/kg BW [11].

Yeast group: Housed and fed basal diet with active dry yeast (Angel Yeast Co., Ltd.) added to concentrate at 0.3% [12].

Farming healthy yaks group: Natural grazing without concentrate supplementation.

1.3 Experimental Diets

Diet formulation referenced the recommended formulas in China's "Beef Cattle Feeding Standards" (NY/T 815-2004), calculated based on an average body weight of 60 kg and daily gain of 400 g. The concentrate primarily consisted of corn, soybean meal, rapeseed meal, wheat bran, rapeseed oil, sodium bicarbonate, salt, and vitamin-mineral premix. The roughage was oat hay. The nutritional levels of the basal diet (not listed as the formula has been patented) were: crude protein (CP) 16.73%, net energy for gain (NEg) 25.19 MJ/kg, calcium (Ca) 0.88%, phosphorus (P) 0.58%, ether extract (EE) 3.79%, neutral detergent fiber (NDF) 34.92%, and acid detergent fiber (ADF) 17.93%.

1.4 Feeding Management

All experimental yaks were uniformly numbered and subjected to immunization and deworming, with other disinfection and immunization procedures following the farm's management protocols. Grazing yaks were kept on natural pasture. All supplementation groups were housed in barns, with 2 yaks from each replicate kept in one pen. Feed was provided twice daily at 08:00 and 16:00. The basal diet was offered based on feed intake measured during the pre-trial period, with a concentrate-to-forage ratio of 35:65 manually mixed into total mixed rations (TMR). Yaks had free access to feed and water, with slight leftovers ensured after each feeding. Residual feed was collected and recorded before the next morning's feeding. Pens were cleaned daily and disinfected weekly.

1.5 Sample Collection

Body measurements were taken on days 1 and 60 before morning feeding. On days 1 and 60, fasting jugular blood samples were collected using vacuum anticoagulant tubes before morning feeding. Samples were divided into two portions: one stored at 4°C for immediate delivery to the laboratory, and another for plasma separation and storage at -20°C for later analysis.

1.6 Measurement Indicators

Body measurement indicators: Body height, body length, chest girth, chest depth, and cannon bone circumference.

Blood routine indicators: Red blood cell (RBC) count, hemoglobin (HGB) content, white blood cell (WBC) count, and platelet (PLT) count were determined using a Japanese SYSMEX-XS-800i hematology analyzer.

Plasma antioxidant indicators: Glutathione peroxidase (GSH-Px) activity, total superoxide dismutase (T-SOD) activity, total antioxidant capacity (T-AOC), malondialdehyde (MDA) content, and catalase (CAT) activity were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

Plasma immune indicators: Immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents were determined by immunoturbidimetry using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.7 Data Processing

Data were analyzed using SPSS 19.0 software. Inter-group comparisons were performed using one-way ANOVA followed by Duncan's multiple comparison test. Comparisons between day 1 and day 60 data were conducted using independent samples t-tests. Results are expressed as mean \pm standard deviation, with $P < 0.05$ considered statistically significant.

Results

2.1 Effects of Different Nutritional Regulations on Body Measurements of Stunted Yaks

As shown in Table 1, on day 1, all stunted yak groups exhibited significantly lower body height, body length, chest girth, and chest depth compared to the farming healthy yaks group ($P < 0.05$). After 60 days of nutritional regulation, the concentrate, cysteamine, and yeast groups showed significantly higher body height, chest girth, chest depth, and cannon bone circumference than the farming yaks with growth retardation group ($P < 0.05$), with no significant differences from the farming healthy yaks group ($P > 0.05$). The average daily gains in chest girth were significantly higher in the concentrate, cysteamine, and yeast groups than in the farming healthy yaks group ($P < 0.05$), while the yeast group also showed significantly higher average daily gains in body height and body length ($P < 0.05$). The concentrate group had significantly greater body length than the farming yaks with growth retardation group ($P < 0.05$) but significantly lower than the cysteamine, yeast, and farming healthy yaks groups ($P < 0.05$). On day 60, the concentrate group showed no significant increase in body length compared to day 1 ($P > 0.05$), while other body measurements were significantly higher ($P < 0.05$). The cysteamine and yeast groups exhibited significant increases in all body measurement indicators from day 1 to day 60 ($P < 0.05$), with significantly higher average daily gains in body length, chest girth, and cannon bone circumference than the farming yaks with growth retardation group ($P < 0.05$), with the yeast group showing the greatest improvement.

2.2 Effects of Different Nutritional Regulations on Blood Routine of Stunted Yaks

As shown in Table 2, on day 60, the concentrate group had significantly higher blood RBC count than the farming yaks with growth retardation group ($P < 0.05$). The concentrate, cysteamine, and yeast groups showed significantly higher blood hemoglobin content than the farming yaks with growth retardation group ($P < 0.05$), with no significant differences from the farming healthy yaks group ($P > 0.05$). The farming yaks with growth retardation group had significantly higher blood WBC count than the other four groups ($P < 0.05$). No significant changes were observed in blood platelet count among all groups ($P > 0.05$). Compared to day 1, blood RBC count on day 60 was significantly lower in both the farming yaks with growth retardation group and the farming healthy yaks group ($P < 0.05$).

2.3 Effects of Different Nutritional Regulations on Plasma Antioxidant Indexes of Stunted Yaks

As shown in Table 3, on day 1, the farming yaks with growth retardation, concentrate, cysteamine, and yeast groups exhibited significantly higher plasma MDA content than the farming healthy yaks group ($P < 0.05$), while their

plasma T-SOD and GSH-Px activities and T-AOC were significantly lower ($P < 0.05$). On day 60, the farming yaks with growth retardation group showed significantly lower plasma T-AOC than on day 1 ($P < 0.05$). The concentrate group had significantly higher plasma T-SOD activity and T-AOC than the farming yaks with growth retardation group ($P < 0.05$). The cysteamine group demonstrated significantly lower plasma MDA content than on day 1 ($P < 0.05$), significantly lower than the farming yaks with growth retardation group ($P < 0.05$), with significantly higher plasma T-SOD activity and T-AOC than day 1 ($P < 0.05$), significantly exceeding the farming yaks with growth retardation group ($P < 0.05$). The yeast group showed significantly lower plasma MDA content than day 1 ($P < 0.05$), significantly lower than the farming yaks with growth retardation group ($P < 0.05$), with significantly higher plasma T-SOD, GSH-Px activities and T-AOC than day 1 ($P < 0.05$), significantly surpassing the farming yaks with growth retardation group ($P < 0.05$).

2.4 Effects of Different Nutritional Regulations on Plasma Immune Indexes of Stunted Yaks

As shown in Table 4, on day 1, the farming yaks with growth retardation, concentrate, cysteamine, and yeast groups had significantly lower plasma IgA and IgG contents than the farming healthy yaks group ($P < 0.05$). On day 60, the concentrate group showed significantly higher plasma IgG content than day 1 ($P < 0.05$), significantly exceeding the farming yaks with growth retardation group ($P < 0.05$). The cysteamine group exhibited significantly higher plasma IgA content than day 1 ($P < 0.05$), significantly higher than the farming yaks with growth retardation group ($P < 0.05$). The yeast group demonstrated significantly increased plasma IgA and IgG contents ($P < 0.05$), significantly surpassing the farming yaks with growth retardation group ($P < 0.05$).

Discussion

3.1 Effects of Different Nutritional Regulations on Body Measurements of Stunted Yaks

Changes in body measurements reflect animal growth and development and are commonly used as indicators to assess animal growth. Due to growth inhibition, stunted yaks have significantly smaller body measurements than normal yaks. Concentrate supplementation can provide the nutrients required for growth in stunted yaks, compensating for nutritional deficiencies and promoting growth. However, after 60 days, the concentrate group showed no significant increase in body length, though chest girth, chest depth, and cannon bone circumference were significantly greater than in the grazing stunted yaks group, yet still smaller than normal yaks. This suggests that merely increasing nutrient intake cannot restore stunted yaks to normal growth levels. Adding cysteamine and active dry yeast to concentrate supplementation further promoted body measurement

growth in stunted yaks. Animal growth is closely related to growth hormone, which is inhibited by somatostatin. Cysteamine can reduce somatostatin activity and increase microbial protein synthesis rates in ruminants, enhancing rumen digestion and metabolism, thereby promoting animal growth and development [13]. Studies have shown that duodenal infusion of cysteamine in Hu sheep increased plasma growth hormone content by 124.2% after 5 days, significantly improving growth rate [14]. Oral administration of cysteamine to sheep increased blood growth hormone content by 56.20% within 6 days, promoting growth [15]. Active dry yeast can improve rumen environment stability, promote rumen microbial fermentation, maintain rumen health, and promote small intestine development, thereby enhancing nutrient digestion and absorption efficiency and promoting growth [16]. The present results indicate that adding active dry yeast to concentrate supplementation produced the greatest improvement in body measurements and the best growth-promoting effects in stunted yaks.

3.2 Effects of Different Nutritional Regulations on Blood Routine of Stunted Yaks

Blood routine indicators reflect nutritional and health status. Red blood cells transport essential substances for metabolism, including oxygen, carbon dioxide, electrolytes, glucose, and amino acids, while also playing a buffering role in acid-base balance. These functions are achieved through hemoglobin in red blood cells [17]. The significant decrease in blood RBC count from day 1 to day 60 in both stunted and normal grazing yaks may be attributed to harsh plateau grazing conditions. The concentrate and cysteamine groups showed significantly higher blood RBC counts than both the grazing stunted yaks group and normal yaks group, while the concentrate, cysteamine, and yeast groups had significantly higher blood hemoglobin content than grazing stunted and normal yaks. These results indicate that concentrate supplementation and the addition of cysteamine and active dry yeast enhanced oxygen transport and metabolic product transport functions, helping to improve metabolism, nutrient status, and growth. White blood cells play important roles in immune function by phagocytizing foreign substances and producing antibodies, which are crucial for wound healing, pathogen defense, and disease resistance [18]. Under traditional grazing conditions, stunted yaks have poorer health status than normal yaks due to adverse environmental factors. The significantly higher blood WBC count in the farming yaks with growth retardation group likely indicates inflammatory responses. However, the addition of cysteamine and active dry yeast maintained WBC counts at levels comparable to normal yaks, suggesting these supplements help enhance immunity and promote healthy, efficient growth in stunted yaks.

3.3 Effects of Different Nutritional Regulations on Plasma Antioxidant Indexes of Stunted Yaks

Cellular function and vitality largely depend on the balance of redox status in the body. When reactive oxygen species (ROS) and free radicals are excessive, the redox balance is disrupted, leading to oxidative damage of cellular macromolecules (lipids, proteins, DNA) and ultimately causing cellular dysfunction or apoptosis [19]. MDA is a product of lipid peroxidation caused by free radical attack on biological membranes, and its content reflects the degree of lipid peroxidation and indirectly indicates cellular damage [20]. GSH-Px in animals uses glutathione (GSH) as a reducing agent to reduce hydrogen peroxide, organic hydroperoxides, and lipid hydroperoxides, helping cells resist oxidative damage [21]. Cells possess endogenous protective mechanisms against oxidative stress through enzymes such as CAT and superoxide dismutase (SOD). T-AOC is a comprehensive indicator reflecting both enzymatic and non-enzymatic antioxidant capacity [22]. The present results indicate that grazing stunted yaks experienced significant oxidative stress compared to normal grazing yaks. Whether oxidative stress inhibits calf growth requires further investigation [23]. The significant decrease in plasma T-AOC in the farming yaks with growth retardation group on day 60 may be due to poor inherent antioxidant capacity combined with harsh plateau grazing conditions. This study found that concentrate supplementation with added cysteamine significantly increased plasma T-SOD activity and significantly decreased plasma MDA content, enhancing antioxidant capacity. Research has shown that cysteamine contains free hydroxyl groups with antioxidant properties that can scavenge free radicals, increase blood GSH content and SOD activity, thereby eliminating free radicals [24]. The present results also demonstrate that concentrate supplementation with active dry yeast significantly increased plasma T-SOD and GSH-Px activities and T-AOC while significantly decreasing plasma MDA content, though the mechanism requires further study. Research indicates that adding yeast culture to sheep diets can increase serum GSH-Px, SOD activities, and T-AOC [25]. Liu et al. [26] reported that dietary yeast supplementation improved antioxidant capacity in cows with subclinical mastitis. Therefore, concentrate supplementation with cysteamine or active dry yeast can enhance antioxidant capacity and alleviate oxidative stress in stunted yaks.

3.4 Effects of Different Nutritional Regulations on Immune Function of Stunted Yaks

The present results indicate that the immune capacity of grazing stunted yaks was significantly lower than that of normal grazing yaks. Concentrate supplementation significantly increased plasma IgG content, likely due to improved nutrient status. Numerous studies have shown that cysteamine can affect immune function directly or indirectly through hormonal changes. On one hand, immune function is closely related to the growth axis. Cysteamine can directly interact with somatostatin, altering its configuration and depleting its immune

activity, thereby relieving somatostatin's inhibitory effect on immunity [27]. On the other hand, cysteamine can relieve somatostatin's inhibition of digestion-related hormones or enzymes, promoting nutrient absorption and utilization, thereby improving immune function [28]. This study demonstrates that concentrate supplementation with added cysteamine significantly increased plasma IgA content, thereby enhancing immune capacity. Yeast has excellent viability and may beneficially affect intestinal flora balance through the rumen, potentially influencing the immune system and animal health [29]. Additionally, organic acids produced by yeast metabolism can lower gastrointestinal pH and effectively inhibit pathogen invasion [30]. Few reports exist on active dry yeast improving immune capacity in ruminants. Studies have shown that feeding yeast preparations can increase *Lactobacillus* counts and decrease *Escherichia coli* counts in goat feces [31], and significantly increase serum IgA and IgG contents in cashmere goats [32]. The present results indicate that concentrate supplementation with active dry yeast significantly increased plasma IgA and IgG contents, thereby enhancing immune capacity in stunted yaks.

Conclusion

Stunted yaks exhibit significantly inferior growth, antioxidant capacity, and immune function compared to normal yaks. Concentrate supplementation, particularly with the addition of 80 mg/kg BW cysteamine or 0.3% active dry yeast, can improve antioxidant and immune capacity while promoting compensatory growth in stunted yaks. The combination of concentrate supplementation with 0.3% active dry yeast produced the most effective results.

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