

Effects of intensive exercise on the blood chemistry of fighting bulls in southern Thailand

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Abstract

Bullfighting is a cultural activity in southern Thailand. Bulls walk and undergo intensive exercise before competition. In the arena, winners are determined by their opponents' surrender, so bulls do not die after fighting. However, there are very few studies on organ injuries in living bulls during these activities. This study compared the effects of walking and intensive exercise on complete blood count (CBC) and blood chemistry. Blood parameters of 14 Thai fighting bulls aged over 6 years, regularly trained for walking and intensive exercise, were measured and analyzed using a paired t-test or Wilcoxon signed-rank test. Eosinophils decreased significantly after intensive exercise ($P < 0.05$), whereas CK, AST, and LDH increased significantly ($P < 0.01$), indicating some degree of muscle damage. Troponin I increased slightly after intensive exercise but remained within the normal range. This study describes only the short-term effects of intensive exercise on CBC and blood chemistry. Long-term effects of intensive exercise or actual fighting warrant further investigation to inform future diagnosis and treatment.

Keywords: biomarker, bull, hemogram, intensive exercise, skeletal muscle injury

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Introduction

Fighting bulls are native to southern Thailand and are used in fighting competitions. There are two types of exercise for fighting bulls: walking exercise and intensive exercise. Bulls with sufficient physical strength engage in competitive fighting behavior in the arena. The winner is determined by the opponent's decision to give up and run away. After fighting, the bulls are treated or allowed to recuperate, then resume walking and intensive exercise to maintain their physical strength and prepare for future competitions.

In humans, intensive exercise causes physiological and/or pathological changes in the short and long term (Boyadjiev and Taralov, 2000; Halson *et al.*, 2003; Belviranli *et al.*, 2017). Many factors affect variation in complete blood count (Telford *et al.*, 2003; McKune *et al.*, 2004; Jukema *et al.*, 2023) and in blood chemistry, especially creatine kinase, including genotype, body composition, and sex; mechanical and metabolic stress; rest-interval length; velocity of eccentric exercise (Koch *et al.*, 2014); running distance (Kim *et al.*, 2009; Son *et al.*, 2015); and intensity of exertion (Saengsirisuwan *et al.*, 1998).

In animals, intensive exercise has been studied most often in horses. Many factors affect the results, as in humans, including the age at which exercise begins (Fan *et al.*, 2002), exercise pattern (Rose *et al.*, 1977; Rose *et al.*, 1980; Serteyn *et al.*, 2010; Mack *et al.*, 2014), exercise intensity and its relationship to exertional rhabdomyolysis (Kristensen *et al.*, 2014; Wanderley *et al.*, 2015; Wilberger *et al.*, 2015), breed effects (Muñoz *et al.*, 2002; Kedzierski and Bergero, 2006), exercise frequency (Lindner *et al.*, 2006), and the effects of age and sex (Harris *et al.*, 1988).

Studies of skeletal muscle injury in fighting bulls are scarce. Spanish fighting bulls that died after fighting showed abnormal blood chemistry associated with muscle damage, including significantly elevated levels of creatine phosphokinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). The elevated marker levels and muscular lesions suggested chronic injury that was exacerbated by the stress of fighting (Purroy and Gonzalez, 1984; Purroy *et al.*, 1992). Because permission from raisers to collect blood samples before actual fighting is difficult to obtain, most research has examined muscle damage in bulls that died after fighting (Alonso *et al.*, 1997; Bartolome *et al.*, 2005; Escalera-Valente *et al.*, 2013; Escalera-Valente *et al.*, 2021). No reports have compared the effects of walking versus intensive exercise in live bulls.

To date, no study has systematically compared hematological and biochemical responses to walking versus intensive exercise in live fighting bulls using a within-animal design. This study aimed to (1) compare hematological responses to walking versus intensive exercise and (2) evaluate biochemical markers of skeletal and cardiac muscle injury. We hypothesized that walking and intensive exercise would elicit distinct hematological and biochemical responses in fighting bulls.

Materials and Methods

Animals: The Institutional Animal Care and Use Committee at Prince of Songkla University approved this study (AUP Code 39/2020). This experiment used fourteen fighting bulls aged six years or older that had undergone regular training in walking and intensive exercise. The bulls were raised on two private farms in Songkhla province. Each raiser cared for only one or two bulls, which resided in the raiser's stable. Daily routine activities included walking 5–7 km in the morning; the bulls were then tethered outdoors with ground anchors for 4–6 h to acclimate them to environmental conditions. Raisers fed the bulls Napier or local grass at approximately 45 kg/day, and water was available *ad libitum*. Bulls were bathed daily, and minor skin lesions were treated as needed.

Bulls underwent intensive exercise at least once a month to assess their readiness for competition in the arena. The strength of a sparring partner was one factor in sparring exercise. For example, if the goal of exercise was to improve strength and fighting skills, the raiser would choose a sparring partner of comparable strength. Alternatively, if raisers wanted to boost a bull's confidence, they would select sparring partners that were younger or weaker. Nevertheless, those younger bulls would also gain experience from sparring with more skilled opponents. This study did not specify the strength of sparring bulls but required each sparring session to last at least 25 minutes and to include protection from a nose brace and horn covering to prevent injury. All fourteen bulls underwent both walking and intensive exercise protocols. No animals were excluded from the analysis due to illness.

Blood collections: Blood was collected twice: the first sample was taken in the evening, 4–6 hours after a normal morning walk, to serve as the baseline. The second sample was collected on the day following intensive exercise, 18–24 hours after exercise, once the animals had completed the required rest period. Blood samples collected in heparinized tubes were tested for red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) using a BC-2800 Vet Auto Hematology Analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China) at the small animal hospital of the Faculty of Veterinary Science, Prince of Songkla University. RBC, WBC, and PLT were measured using the impedance method, and HGB was measured using the colorimetric method. HCT, MCV, MCH, and MCHC were calculated automatically. White blood cell differentials were estimated from a WBC histogram.

Blood samples in non-heparinized tubes were left to coagulate for 15 min, then centrifuged to obtain serum. Serum samples were used to assess skeletal and cardiac muscle damage by measuring AST, CK, serum myoglobin, and Troponin-I via chemiluminescence, and to assess liver and kidney function by measuring AST, GGT, BUN, and creatinine (CR) using the Cobas® 6000 analyzer series (Roche Diagnostics Co. Ltd., USA).

at the N-Health laboratory in Songkhla, Thailand. The lower limit of detection for Troponin-I was 0.0019 ng/mL.

Statistical analysis: A dependent paired t-test was used to compare quantitative data between the two experimental groups. Normality was assessed using the Kolmogorov-Smirnov test; when this assumption was violated, the Wilcoxon signed-rank test was used instead. All statistical analyses were performed using Statistics Kingdom (a free online program; <https://www.statskingdom.com>). All statistical tests were two-tailed, with significance set at $P < 0.05$ and high significance set at $P < 0.01$.

Results

The leucogram was similar across both exercise types, except for eosinophils, which were $1.07 \pm 0.47 \times 10^3$ cells/mm³ during intensive exercise, significantly lower than during walking exercise ($1.45 \pm 0.78 \times 10^3$ cells/mm³; $P = 0.048$) (Table 1).

The BUN value for intensive exercise was 11.87 ± 3.99 mg/dL, not significantly different from that for walking exercise (10.33 ± 3.54 mg/dL; $P = 0.052$). The CR value for intensive exercise was 2.24 ± 0.26 mg/dL,

also not significantly different from that for walking exercise (2.26 ± 0.32 mg/dL; $P = 0.705$) (Table 2).

The CK, AST, and LDH levels in the intensive exercise group were significantly higher than those in the walking exercise group (872.86 ± 761.39 vs. 242.50 ± 243.51 U/L; $P = 2.441 \times 10^{-4}$ for CK, 121.71 ± 45.76 vs. 80.93 ± 24.91 U/L; $P = 2.219 \times 10^{-3}$ for AST, and 1527.21 ± 259.59 vs. 1228.64 ± 192.33 U/L; $P = 5.362 \times 10^{-5}$ for LDH) (Table 3).

GGT levels in the intensive and walking exercise groups were not significantly different (28.21 ± 22.01 vs. 22.00 ± 9.80 ; $P = 0.721$) (Table 3).

Serum myoglobin levels were lower than 21 ng/mL in both groups (below the detection limit of the Cobas® 6000 analyzer). Because the equipment's minimum detection limit for serum myoglobin, statistical analysis could not be performed; therefore, the results are presented descriptively.

For troponin I, only 5 of the walking exercise bulls had values above the detection limit (0.0019 ng/mL), whereas 12 of the intensive exercise bulls had values above this limit. These values remained within the normal range reported previously (Table 4).

Table 1 Complete blood count between walking and intensive exercise compared to previous reports.

Parameter	Walking exercise (Mean $\pm$ SD)	Intensive exercise (Mean $\pm$ SD)	P-value	References			
				Khophloiklang <i>et al.</i> , 2019	Krimer, 2011	Radostits <i>et al.</i> , 2006	Constable <i>et al.</i> , 2017
WBC ($\times 10^3$ /mm ³) [†]	8.33 ± 1.79	8.28 ± 1.79	0.525	9.08 ± 2.38	4.0 – 12.0	4.0 – 12.0	4.9 – 12.0
Segmented neutrophil (10^3 /mm ³) [†]	3.57 ± 1.59	4.04 ± 1.06	0.194	4.43 ± 1.92	0.6–4.0	0.6 – 4.0	1.8 – 6.3
Band neutrophil (10^3 /mm ³) [†]	0.12 ± 0.39	0.00 ± 0.00	-	-	0.0–0.1	0.0 – 0.12	Rare
Lymphocyte (10^3 /mm ³) [†]	2.72 ± 1.24	2.94 ± 0.80	0.653	3.08 ± 1.29	2.5–7.5	2.5 – 7.5	1.6 – 5.6
Monocyte (10^3 /mm ³) [†]	0.43 ± 0.31	0.25 ± 0.21	0.092	0.45 ± 0.21	0.0–0.9	0.0 – 0.8	0 – 0.8
Eosinophil (10^3 /mm ³) [†]	1.45 ± 0.78	1.07 ± 0.47	0.048	1.14 ± 0.72	0.0–2.4	0.0 – 2.4	0 – 0.9
Basophil (10^3 /mm ³) [†]	0.02 ± 0.06	0.01 ± 0.03	0.437	-	0.0–0.2	-	-
RBC (10^6 /mm ³) [†]	6.84 ± 0.80	6.47 ± 0.94	0.263	7.17 ± 0.97	5.0–10.0	5.0 – 10.0	5.1 – 7.6
HCT (%) [†]	38.50 ± 4.73	36.88 ± 6.38	0.589	39.55 ± 5.42	24–46	24 – 46	22 – 33
HGB (g/dL) [†]	13.80 ± 1.50	13.32 ± 1.96	0.221	13.21 ± 1.68	8.0–15.0	8.0 – 15.0	8.5 – 12.2
MCV (fl) [†]	57.68 ± 4.37	57.85 ± 5.21	0.606	55.33 ± 4.60	40 – 60	40 – 60	38 – 50
MCH (pg) [†]	20.23 ± 1.59	20.67 ± 2.11	0.377	18.49 ± 1.31	11 – 17	11 – 17	14 – 18
MCHC (g/dL) [†]	35.12 ± 1.91	35.75 ± 1.38	0.429	33.52 ± 2.01	30 – 36	30 – 36	36 – 39
PLT (10^3 /mm ³) [†]	195.14 ± 154.22	198.38 ± 124.06	0.704	283.36 ± 97.88	100–800	-	-
Plasma protein (g/100 mL) [†]	8.61 ± 0.69	8.40 ± 0.74	0.322	-	6.0–8.0	5.7 – 8.1	5.7 – 8.1

[†] Dependent pair t-test.

[‡] Wilcoxon signed-rank test.

Discussion

RBC, HCT, HGB, MCV, MCH, and MCHC did not differ significantly between the two types of exercise ($P > 0.05$). This contrasts with findings in human athletes, where decreases in RBC, HCT, and HGB are commonly observed and attributed to several factors, including exercise-induced intra- and extravascular hemolysis due to impact forces - a condition known as sports anemia (Damian *et al.*, 2021).

The eosinophil count in the intensive exercise group was significantly lower than in the walking

exercise group ($P = 0.048$). This exercise-induced decrease in eosinophils is also consistent with the findings of Sedeño *et al.* (2025). This mirrors findings in humans, in whom eosinophils decrease after exercise and return to normal levels within 24 h (Jukema *et al.*, 2023; Spijkerman *et al.*, 2021). Eosinophils can migrate from blood vessels into inflammatory tissue via increased CD11b (a migration molecule) and decreased CD62L (an adhesion molecule) on the cell membrane (Jukema *et al.*, 2023).

Table 2 BUN and CR levels between walking and intensive exercise compared to previous reports.

Conditions	BUN (mg/dL) [†] (Mean ± SD)	CR (mg/dL) [†] (Mean ± SD)
Walking exercise	10.33 ± 3.54	2.26 ± 0.32
Intensive exercise	11.87 ± 3.99	2.24 ± 0.26
P-value	0.052	0.705
References from healthy cattle		
Boonprong <i>et al.</i> , 2007		
- in Thai indigenous cattle	29.68 ± 0.84	1.60 ± 0.04
- in Simmental x Brahman	25.48 ± 1.12	1.60 ± 0.07
Constable <i>et al.</i> , 2017	5.6 – 26.8	1 – 1.98
Kaneko <i>et al.</i> , 2008	20 – 30	1.0 – 2.0
Krimer, 2011	10 – 25	0.5 – 2.2
Otter, 2013	9.52 – 20.44	0.5 – 1.87
Radostits <i>et al.</i> , 2006	6.0 – 27	1.0 – 2.0
Reference from fighting bull		
Alonso <i>et al.</i> , 1997*	16.24	3.14
Bartolome <i>et al.</i> , 2005*	39.2	3.6
Escalera-Valente <i>et al.</i> , 2021	16.61 ± 3.56	2.68 ± 0.00

[†] Dependent pair t-test.

* Wilcoxon signed-rank test.

* Referred from Escalera-Valente *et al.*, 2021.**Table 3** CK, AST, GGT, LDH, and serum myoglobin levels between walking and intensive exercise compared to previous reports.

Conditions	CK (U/L) [†] (Mean ± SD)	AST (U/L) [†] (Mean ± SD)	GGT (U/L) [†] (Mean ± SD)	LDH (U/L) [†] (Mean ± SD)	Serum myoglobin (ng/mL)
Walking exercise	242.50 ± 243.51	80.93 ± 24.91	22.00 ± 9.80	1228.64 ± 192.33	<21
Intensive exercise	872.86 ± 761.39	121.71 ± 45.76	28.21 ± 22.01	1527.21 ± 259.59	<21
P value	2.441 x 10 ⁻⁴	2.219 x 10 ⁻³	0.721	5.362 x 10 ⁻⁵	-
Reference from healthy cattle					
Boonprong <i>et al.</i> , 2007					
- In Thai indigenous cattle	209.5 ± 12.4	81.2 ± 1.9	17.0 ± 0.7	-	-
- In Simmental x Brahman	192.8 ± 18.7	68.6 ± 2.4	14.5 ± 0.7	-	-
Constable <i>et al.</i> , 2017	35 – 280	78 – 132	6.1 – 17.4	692 – 1455	-
Kaneko <i>et al.</i> , 2008	4.8 – 12.1	78 – 132	6.1 – 17.4	692 – 1445	-
Krimer, 2011	0 – 350	60 – 125	6 – 17.4	-	-
Otter, 2013	0 – 200	75 – 130	0 – 30	-	-
Radostits <i>et al.</i> , 2006	35 – 280	78 – 132	6.1 – 17.4	692 – 1445	-
Osame and Ichijo, 1987	-	-	-	-	<10-80
Reference from the fighting bull					
Alonso <i>et al.</i> , 1997*	3,139.1	318.5	178.1	-	-
Bartolome <i>et al.</i> , 2005*	1,582.8	171.1	178.1	-	-
Escalera-Valente <i>et al.</i> , 2021	6,729 ± 10931	495 ± 462	50 ± 34	2,828 ± 1,975	-
Purroy and Gonzalez, 1984					-
- Normal strength during fighting	1,041 ± 501	-	-	3,013 ± 627	-
- Low strength during fighting	1,916 ± 568	-	-	3,604 ± 589	-
- Very low strength during fighting	5,152 ± 3,884	-	-	4,838 ± 2346	-
Purroy <i>et al.</i> , 1992	2,162 ± 2,006	149 ± 86	-	2,897 ± 861	-

[†] Dependent pair t-test.

* Wilcoxon signed-rank test.

* Referred from Escalera-Valente *et al.*, 2021.

Table 4 Troponin I levels between walking and intensive exercise compared to previous reports (lower limit of troponin test in this experiment is 0.0019 mg/mL).

Conditions	Mean (ng/mL)	Median (ng/mL)	Range (ng/mL)	References
Walking exercise	-	-	≤ 0.0019 (n = 9) > 0.0019 – 0.0059 (n = 5)	-
Intensive exercise	-	-	≤ 0.0019 (n = 2) > 0.0019 – 0.0223 (n = 12)	-
<i>Previous report</i>				
Normal cattle	-	0.00	0.00 – 0.01	Varga et al., 2013
	-	-	0.00 – 0.036	Labonte et al., 2015
	0.17 (only male)	-	0.00 – 0.19	Basbugan et al., 2010
	0.18 (over all)	-	0.00 – 0.23	
	0.0204 ± 0.011	-	-	Venkatesan et al., 2020
	0.0228 ± 0.002	0.02	0.01–0.04	Basak et al., 2015
Metritis	-	0.01	0.00 – 1.90	Varga et al., 2013
Mastitis	-	0.03	0.01 – 0.15	Varga et al., 2013
Peritonitis	-	0.05	0.01 – 0.18	Varga et al., 2013
LDA	-	0.04	0.01 – 0.20	Varga et al., 2013
LDA and metritis	-	0.09	0.02 – 0.29	Varga et al., 2013
Pneumonia	-	0.03	0.00 – 0.05	Varga et al., 2013
Dystocia requires C-section	-	0.08	0.04 – 1.20	Varga et al., 2013
Downer cow syndrome	-	1.70	0.15 – 27.00	Varga et al., 2013
Moninsin toxicity	-	16.00	0.40 – 39.00	Varga et al., 2009
Theileria infection	0.028	-	0.005 – 0.21	Fartashvand et al., 2013
FMD				Aly et al., 2020
- Healthy calve	0.035 ± 0.002	-	-	
- < 3 months calve	2.89 ± 0.23	-	-	
- > 3-6 months calve	2.19 ± 0.02	-	-	
Traumatic Reticulo- Pericarditis	0.10 ± 0.034	-	-	Venkatesan et al., 2020
Bovine respiratory disease				Basak et al., 2015
- Healthy calves	0.0228 ± 0.002	0.02	0.01 – 0.04	
- Moderately diseased calves	0.026 ± 0.002	0.02	0.01– 0.04	
- Severely diseased calves	0.308 ± 0.156	0.1	0.04 – 2.84	

The AST values of the intensive exercise bulls were also within the normal range (Krimer, 2011) but were significantly higher than those of the walking exercise bulls ($P = 2.219 \times 10^{-3}$). AST is less sensitive than CK, which may explain why AST levels in bulls undergoing intensive exercise remained within the normal range; alternatively, the timing of blood collection may not have been optimal for accurate AST evaluation.

The LDH value for intensive exercise exceeded the reference value for normal cattle (Table 3). This study showed a highly significant increase in LDH during intensive exercise compared with walking exercise ($P = 5.362 \times 10^{-5}$); however, we cannot conclude whether this reflects true muscle damage or other contributing factors (Anderson, 1976). LDH is a non-specific indicator of tissue damage in cattle, as it can be released in response to many conditions, including breed, age, stress, exercise, sex, season, liver, respiratory, or muscle disease, metabolic disease, feeding system, milk production, and intoxication (Klein et al., 2020). During exercise, LDH may increase due to anaerobic glycolysis, metabolic demand, or direct leakage from damaged muscle (Lee et al., 2011).

A study of blood collected immediately after bulls died following fights with matadors found that increases in CK, LDH, and especially AST resulted from heavy exercise before the fight, rather than the fight itself, since there was not enough time for AST to increase during the fight (Purroy et al., 1992). Most of the fighting bulls (78%) had histological lesions in skeletal or cardiac muscle, with a predominance of chronic lesions (Purroy et al., 1992).

GGT does not directly indicate skeletal muscle damage but rather reflects liver and bile duct function. It is elevated in fighting bulls that engaged in actual combat and died (Alonso et al., 1997; Bartolom et al., 2005; Escalera-Valente et al., 2021). This may be due to the stress-induced rise in cortisol levels, which directly induces GGT synthesis in hepatocytes, as demonstrated in laboratory animal models (Barouki et al., 1983), or to the relatively high energy expenditure during exercise, which affects metabolic processes and increases oxidative stress in the liver (Escribano et al., 2010).

In our experiment, both walking and intensive exercise resulted in higher GGT values than normal cattle (Kaneko et al., 2008; Krimer, 2011; Radostits et al., 2006), although the values remained within the normal

range reported by Otter (2013). However, our data were lower than those in bulls that died from actual combat, as mentioned above. In addition, there was no statistically significant difference between walking and intensive exercise in this experiment. This suggests that walking and intensive exercise influence GGT levels to some extent, but not as much as actual combat.

Serum myoglobin remained within the normal range for healthy cattle (<10–80 ng/mL) (Osame and Ichijo, 1987), although CK and AST increased. This may reflect a low level of muscle injury, or the short half-life of serum myoglobin (2–3 h), which returns to normal by 6 to 8 hours. Myoglobin may therefore be a useful marker for more severe muscle injury, such as that occurring during actual fighting, and for monitoring the treatment of rhabdomyolysis (Raju et al., 2017).

BUN and CR were used to assess kidney function. BUN levels for walking exercise and intensive exercise were not significantly different ($P = 0.052$) and remained within the standard reference range (10–25 mg/dL) (Krimer, 2011). This is consistent with the absence of dehydration symptoms in both groups of cattle.

The CR values for walking exercise and intensive exercise were not significantly different ($P = 0.705$); however, both were at the upper end of the standard reference range (0.5–2.2 mg/dL) (Krimer, 2011). Creatinine is a product of muscle metabolism, and its daily excretion is related to muscle mass in cattle (Costa e Silva et al., 2014) and to muscle activity affecting kidney injury markers in humans (Bongers et al., 2017).

Many studies have examined the use of troponin-I to assess cardiac muscle damage in cattle with various diseases. This study found that intensive exercise produced slightly higher troponin-I values than walking exercise, although these values remained within the normal range reported in cattle (Basak et al., 2015; Basbugan et al., 2010; Labonte et al., 2015; Venkatesan et al., 2020). This slight elevation may indicate a low level of cardiac muscle injury during intensive exercise. However, this laboratory test could not measure troponin-I levels below 0.0019 ng/mL, which limited our ability to determine the magnitude of troponin-I change in each bull.

In conclusion, the intensive exercise group showed a significantly lower eosinophil count than the walking exercise group, a pattern consistent with previous findings in both cattle and humans, where eosinophils decrease after exercise. In humans, this decrease may be explained by the migration of eosinophils from blood vessels into inflammatory tissue, driven by increased expression of the migration molecule CD11b and decreased expression of the adhesion molecule CD62L on the cell membrane.

Walking exercise bulls had average CK values within the normal range but with high standard deviations, suggesting that walking exercise may cause mild or variable muscle damage in some individuals. The average CK value after intensive exercise was significantly higher than that after walking exercise, indicating that intensive exercise caused more

pronounced muscle damage. The AST value after intensive exercise was also significantly higher than that after walking exercise, although AST values for both types of exercise remained within the normal range; this may be because AST is less sensitive to skeletal muscle damage than CK. The average LDH value after intensive exercise was significantly higher than that after walking exercise, which may reflect a metabolic response, greater muscle damage, or both.

A limitation of this study is the mismatch in the timing of sample collection after exercise. The raisers changed the original plan and declined blood collection 4–6 hours after intensive exercise, as they believed the bulls would perceive this as punishment; they did not want to cause the bulls additional pain and preferred that the animals rest first, so blood collection was instead permitted on the following day. This mismatch likely affected the statistical calculations, since CK and AST differ in their latent period, rising time, peak time, half-life, and clearance kinetics. However, we considered that daily walking exercise would result in relatively stable CK and AST levels; therefore, bulls undergoing walking exercise were designated as the control group. Bulls undergoing intensive exercise underwent a single bout of strenuous exercise every 1–2 months, and the two groups were compared statistically.

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