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Blood mineral profile of Kacang and Jawarandu goats grazing intact and degraded karst limestone pastures compared with non-karst controls: a 12-month longitudinal study at Kawasan Karst Gombong Selatan (KKGS), Kebumen, Central Java, Indonesia

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Abstract

Karst limestone mountain ecosystems are characterised by alkaline calcareous substrates (pH 7.5–8.5) that substantially alter soil mineral bioavailability. Despite the Kawasan Karst Gombong Selatan (KKGS) in Kebumen, Central Java, Indonesia, supporting extensive goat farming across Kecamatan Ayah, Buayan, and Rowokele, no study has quantified the blood mineral profile of goats grazing this ecosystem. This study established the first blood mineral baseline (Ca, P, Mg, Fe, Zn, Cu) and production parameters (average daily gain/ADG, body condition score/BCS, haemoglobin/Hb) of Kacang and Jawarandu goats on intact (KKGS-IK, Kec. Ayah) and degraded (KKGS-DK, Kec. Buayan) karst pastures versus non-karst controls (NK, Kec. Rowokele) over 12 months. A randomised comparative cohort (n = 90; 3 groups x 2 breeds x 15/cell) collected blood at months 0, 3, 6, 9, and 12. Concurrent soil and forage mineral analyses were performed. Data were analysed by one-way ANOVA, Tukey HSD post-hoc, Pearson correlation, and Cohen's *d* effect size. Goats on intact karst showed significantly higher serum Ca (Kacang: 11.40 +/- 1.04 vs. 8.38 +/- 0.79 mg/dL; Jawarandu: 11.95 +/- 0.97 vs. 8.72 +/- 0.87 mg/dL; $p < 0.001$; $d > 2.97$) and Mg ($p < 0.001$; $d = 3.28$), but significantly lower blood Fe (-23.1%; $p < 0.001$; $d = -2.17$) than non-karst controls. Forage Ca:P ratio in karst legumes (6.71–9.73:1) substantially exceeded the NRC (2007) recommended range (1.5:1-2.5:1), resulting in lower blood P in karst goats ($p < 0.01$; $d = -0.70$). ADG was 35.4% higher in intact karst ($p < 0.001$; $d = 3.01$). Blood Ca and Mg correlated positively with ADG ($r = 0.580$ and 0.595; $p < 0.001$). KKGS intact karst provides a natural Ca and Mg advantage eliminating mineral block supplementation needs, but creates a clinically relevant Ca:P imbalance and Fe reduction requiring targeted management.

Introduction

Karst limestone mountain ecosystems–geological formations produced by the dissolution of calcium carbonate (CaCO3) bedrock–cover approximately 15–20% of Earth's land surface and profoundly alter the geochemical environment available to grazing livestock (Ford and Williams 2007). At alkaline soil pH (7.5–8.5) characteristic of calcareous substrates, soluble calcium (Ca) and magnesium (Mg) concentrations are substantially elevated, while iron (Fe), zinc (Zn), and phosphorus (P) availability are reduced through formation of insoluble complexes (Haenlein and Anke 2011). These geochemical properties create a nutritional environment for ruminants that differs fundamentally from conventional pasture soils and requires dedicated characterisation.

The Kawasan Karst Gombong Selatan (KKGS) is a Miocene-age (Formasi Kalipucang, 11–25 Ma) cockpit-type karst system located in the southwestern corner of Kebumen Regency, Central Java Province (7 degrees 40'-7 degrees 48'S, 109 degrees 26'-109 degrees 41'E), encompassing Kecamatan Ayah, Buayan, and Rowokele. Designated as a Protected Karst Area (Kawasan Lindung) by Presidential Decree in 2004 and integrated into Geopark Kebumen, KKGS spans > 40 km2 with 182 caves including Gua Petruk (> 2 km) and two karst lakes (kebumenkab.go.id 2015). Mean annual rainfall is approximately 2,400 mm. Dominant browse vegetation accessible to goats includes *Leucaena leucocephala, Indigofera zollingeriana*, and *Desmodium* spp.--adapted to calcareous substrates with crude protein (CP) of 21–27% DM and Ca:P ratios of 6–10:1.

Goats (*Capra hircus*) are the primary livestock species in KKGS, with estimated populations of > 85,000 animals across the three kecamatan (BPS Kebumen 2023), predominantly comprising Kacang (Indonesia's most widely distributed indigenous breed) and Jawarandu (Kacang x Ettawa cross). A global meta-analysis (Sarwanto 2025) synthesising 34 goat-specific studies from 18 countries identified karst grazing as conferring significant blood Ca advantages (Hedges' d = + 0.68; $p < 0.001$) while reducing blood Fe (d = -0.71; $p < 0.001$), but no Indonesian data contributed. This study addresses these gaps with the objectives of: (i) establishing the first blood mineral baseline (Ca, P, Mg, Fe, Zn, Cu) and production parameters (ADG, BCS, Hb) for Kacang and Jawarandu goats grazing intact and degraded KKGS pastures versus non-karst controls; (ii) characterising soil and forage mineral composition at all sites; (iii) assessing breed-specific blood mineral differences; (iv) determining longitudinal mineral trends over 12 months; and (v) formulating evidence-based mineral management recommendations for KKGS goat farmers.

Materials and Methods

Ethical approval

All animal procedures were approved by the Animal Care and Use Committee (ACUC) of Universitas Wijayakusuma Purwokerto (Approval No.: ACUC-UNWIKU-2024-018) and comply with the ARRIVE 2.0 reporting guidelines (Percie du Sert et al. 2020). Informed written consent was obtained from all participating farmers.

Study sites and design

The study was conducted January-December 2024 at three sites in Kebumen Regency (Fig. 1): Site 1 -- **KKGS-IK (Intact Karst)**, Kecamatan Ayah (7 degrees 43'S, 109 degrees 29'E; elevation 180-350 m asl; bare rock <10%; soil pH 7.82; dominant vegetation: *L. leucocephala, I. zollingeriana, Desmodium* spp., native karst grass); Site 2 -- **KKGS-DK (Degraded Karst)**, Kecamatan Buayan (7 degrees 44'S, 109 degrees 34'E; elevation 150-320 m asl; bare rock 25-50%; pH 7.45; sparse pioneer grasses); Site 3 -- **Non-Karst Control (NK)**, Dataran Rowokele, Kecamatan Rowokele (7 degrees 41'S, 109 degrees 43'E; elevation 35-60 m asl; pH 6.12; *Brachiaria mutica, Panicum maximum*, Napier grass). Ninety clinically healthy goats (45 Kacang, initial BW 15.8 +/- 2.1 kg; 45 Jawarandu, 20.4 +/- 2.8 kg; age 8-12 months) were randomly allocated to three groups (n = 30/group: 15 Kacang + 15 Jawarandu). Animals grazed naturally without supplemental feeding.

Blood sampling and mineral analysis

Blood samples (10 mL) were collected from the jugular vein at months 0, 3, 6, 9, and 12 (07:00-09:00 h, 12 h post-prandial). Serum was separated by centrifugation (3,000 rpm x 15 min) and stored at -20 degrees C. Mineral analyses were conducted at the Agricultural Laboratory, Universitas Wijayakusuma Purwokerto: Ca, Mg, Fe, Zn (flame atomic absorption spectrophotometry, AAS; Shimadzu AA-6300); P (colorimetric molybdovanadate; Shimadzu UV-1800). Haemoglobin (Hb) was determined by the cyanmethaemoglobin method (HumaLyzer 3000). Body condition score (BCS) was assessed on a 1-5 scale (Dethan et al. 2019). ADG was calculated from monthly weighing.

Soil and forage analysis

Composite soil samples (0-20 cm; n = 10 subsamples/site) were collected at months 0, 6, and 12 and analysed at the Soil Science Laboratory, Universitas Wijayakusuma Purwokerto: pH (1:5 H2O); organic matter (Walkley-Black); cation exchange capacity (CEC, ammonium acetate); Ca, Mg, Fe, Zn, Cu (AAS after aqua regia digestion). Forage samples (six dominant species/site; n = 5 composite samples) were collected at 45-day intervals and analysed for: DM (105 degrees C); CP (Kjeldahl x 6.25); NDF and ADF (Van Soest et al. 1991); Ca, P, Mg, Fe (AAS or colorimetric after dry ashing).

Artificial intelligence disclosure

In accordance with Springer Nature editorial policy, the authors declare that Claude (Anthropic, version 3.x) was used for AI-assisted copy editing to improve readability, grammar, and style of human-authored text. No AI tool generated substantive scientific content, data, analyses, or conclusions. All intellectual content, experimental design, data collection, statistical analysis, and interpretations are the sole work of the authors, who take full responsibility for the accuracy and integrity of the published work.

Statistical analysis

One-way ANOVA tested group effects at Month 6 (primary endpoint), with Tukey HSD post-hoc pairwise comparisons. Effect size was estimated as eta-squared (eta2) from ANOVA and Cohen's *d* for pairwise comparisons (Cohen 1988: d = 0.2 small; 0.5 medium; 0.8 large). Longitudinal data were analysed by linear mixed-effects model (LME; lme4 package, R v4.3.1) with group, breed, month, and group x month interaction as fixed effects, and animal ID as random effect. Pearson correlations were computed between blood minerals and production parameters. Significance threshold: alpha = 0.05.

Results

Soil mineral profile and forage composition

Soil pH differed markedly across sites (KKGS-IK: 7.82; KKGS-DK: 7.45; NK: 6.12; $p < 0.001$; Table 1). Soil Ca was 3.65-fold higher at KKGS-IK (2.84%) than at NK (0.61%), and soil Mg followed a parallel gradient (0.41% vs. 0.17%). Conversely, soil Fe and Zn were highest at NK (182 and 71 ppm, respectively) and lowest at KKGS-IK (145 and 42 ppm), consistent with precipitation of these micronutrients as insoluble hydroxides at alkaline pH. Soil organic matter was lowest at KKGS-DK (1.62%), reflecting vegetation loss from karst degradation.

Forage Ca:P ratio in KKGS legumes substantially exceeded the NRC (2007) recommended range of 1.5:1-2.5:1 (Table 2; Fig. 4b): *L. leucocephala* (9.73:1), *l. zollingeriana* (6.71:1), *Desmodium* sp. (6.88:1). Non-karst grasses (*B. mutica*: 1.71:1; *P. maximum*: 1.68:1) were within the recommended range. Forage Fe was highest in non-karst grasses (284-318 ppm DM) and lowest in KKGS legumes (148-204 ppm DM), reflecting soil Fe availability patterns (Fig. 4a).

Table 1 Soil mineral composition and physical properties at three study sites in Kebumen Regency, Central Java (mean +/- SD; n = 10 composite samples/site)

Parameter	Unit	KKGS-IK	KKGS-DK	Non-Karst Control	F	Sig.
Soil pH	--	7.82+/-0.14a	7.45+/-0.18b	6.12+/-0.22c	284.6	***
Organic matter	%	2.84+/-0.42b	1.62+/-0.31c	3.21+/-0.48a	48.2	***
CEC	cmol(+)/kg	24.8+/-2.1a	18.4+/-2.6b	16.2+/-1.9b	64.8	***
Ca	%	2.84+/-0.38a	1.62+/-0.29b	0.61+/-0.14c	312.4	***
P	%	0.062+/-0.008a	0.058+/-0.007a	0.044+/-0.006b	28.4	***
Mg	%	0.41+/-0.06a	0.28+/-0.05b	0.17+/-0.04c	158.6	***
Fe	ppm	145+/-18c	168+/-22b	182+/-24a	24.1	***
Zn	ppm	42+/-6c	56+/-8b	71+/-9a	62.4	***
Bare rock	%	5.2+/-2.1	38.4+/-6.8	0.0	--	--

*KKGS-IK = intact karst (Kec. Ayah); KKGS-DK = degraded karst (Kec. Buayan); CEC = cation exchange capacity. Different superscript letters (a, b, c) within rows indicate Tukey HSD significance ($p < 0.05$). *** $p < 0.001$*

Table 2 Nutritive value and mineral composition of dominant forage species at KKGS and non-karst sites (DM basis; mean +/- SD; n = 5 composite/species)

Species	Site	CP (%)	NDF (%)	Ca (%)	P (%)	Fe (ppm)	Ca:P	NRC status
I. zollingeriana	KKGS	26.8+/-1.2	38.4+/-2.1	1.88+/-0.14	0.28+/-0.02	182+/-18	6.71:1	>>Max
L. leucocephala	KKGS	23.4+/-1.8	36.2+/-1.9	2.14+/-0.18	0.22+/-0.02	148+/-14	9.73:1	>>Max
Desmodium sp.	KKGS	21.8+/-2.1	42.1+/-2.4	1.65+/-0.12	0.24+/-0.02	204+/-22	6.88:1	>>Max
Native karst grass	KKGS	8.4+/-1.4	68.4+/-3.8	0.84+/-0.08	0.18+/-0.02	242+/-28	4.67:1	>Max
B. mutica	NK	9.1+/-1.2	64.8+/-3.2	0.48+/-0.06	0.28+/-0.03	284+/-32	1.71:1	Normal
P. maximum	NK	11.2+/-1.6	62.3+/-3.0	0.52+/-0.06	0.31+/-0.03	318+/-36	1.68:1	Normal

DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; NRC status: comparison with NRC (2007) recommended Ca:P (1.5:1-2.5:1); >>Max = greatly exceeds; >Max = exceeds; Normal = within range

Blood mineral profile at Month 6

One-way ANOVA revealed significant group effects for all eight measured variables (all $p < 0.001$; Table 3). Effect sizes (eta2) were very large for Ca (eta2 = 0.617), Mg (eta2 = 0.605), ADG (eta2 = 0.584), and Fe (eta2 = 0.485), indicating that karst ecosystem condition explained 49-62% of total variance. The complete blood mineral profile is presented in Fig. 2.

Table 3 Blood mineral concentrations, haemoglobin, BCS, and ADG of Kacang and Jawarandu goats at Month 6 by treatment group (mean +/- SD; n = 15/cell)

Group	Breed	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	Fe (ug/dL)	Hb (g/dL)	BCS	ADG (g/d)
KKGS-IK	Kacang	11.40+/-1.04a	5.55+/-0.50ab	2.88+/-0.19a	107.84+/-11.78b	11.49+/-1.14ab	3.18+/-0.23a	108.97+/-15.29a
	Jawarandu	11.95+/-0.97a	5.27+/-0.56bc	2.88+/-0.38a	121.75+/-14.50ab	11.74+/-0.65a	3.37+/-0.39a	117.56+/-10.72a
KKGS-DK	Kacang	8.59+/-0.97b	4.84+/-0.58c	2.42+/-0.21b	110.47+/-11.78b	9.78+/-1.06c	2.83+/-0.28b	78.42+/-15.68b
	Jawarandu	9.83+/-0.94b	5.13+/-0.69bc	2.21+/-0.28b	125.63+/-16.42a	10.03+/-1.04bc	2.96+/-0.31b	88.33+/-16.24b
NK	Kacang	8.38+/-0.79b	5.83+/-0.86a	1.90+/-0.18c	151.08+/-15.42a	11.41+/-0.95ab	2.64+/-0.31b	74.43+/-13.84b
	Jawarandu	8.72+/-0.87b	5.93+/-0.68a	1.94+/-0.22c	147.63+/-19.06a	11.73+/-0.92a	2.78+/-0.35b	80.25+/-14.58b
ANOVA	**F**	**70.16*** **	**13.78*** **	**66.62*** **	**40.89*** **	**22.70*** **	**13.65*** **	**61.13*** **
	eta2	**0.617**	**0.241**	**0.605**	**0.485**	**0.343**	**0.239**	**0.584**
NRC (2007)	**Range**	**8.0-12.0**	**4.5-7.5**	**1.8-3.5**	**100-200**	**8.0-14.0**	**--**	**>75**

*Different superscript letters (a, b, c) in columns indicate Tukey HSD significance ($p < 0.05$). *** $p < 0.001$. eta2 = eta-squared effect size. BCS = body condition score (1-5 scale). ADG = average daily gain. KKGS-IK = intact karst (Kec. Ayah); KKGS-DK = degraded karst (Kec. Buayan); NK = non-karst control (Kec. Rowokele)*

Calcium and magnesium

Serum Ca in KKGS-IK goats was 37-38% higher than non-karst controls (Kacang: +36.2%; Jawarandu: +38.0%; $p < 0.001$; Cohen's $d > 2.97$). Both breeds maintained Ca within the NRC (2007) normal range (8.0-12.0 mg/dL). Blood Mg was 50% higher in intact karst vs. controls ($d = 3.28$; $p < 0.001$), with all KKGS-IK values well exceeding the NRC minimum (1.8 mg/dL). Non-karst controls (1.90-1.94 mg/dL) were near the minimum threshold, indicating hypomagnesaemia risk on grass-dominated pastures.

Phosphorus and Ca:P imbalance

Blood P in KKGS-IK goats was significantly lower than in non-karst controls ($p < 0.01$; $d = -0.70$), though all values remained within the NRC normal range (4.5-7.5 mg/dL). KKGS-DK Kacang showed the lowest blood P (4.84 +/- 0.58 mg/dL), approaching the lower boundary. The dietary Ca:P ratios of 6.71-9.73:1 in KKGS legumes drove competitive inhibition of intestinal P absorption through Ca2+-HPO42- competition at the NaPi-IIb transporter (Zillinger et al. 2024).

Iron, zinc, and haemoglobin

Blood Fe was significantly reduced in KKGS-IK goats compared with non-karst controls (overall -23.1%; $p < 0.001$; $d = -2.17$). A progressive longitudinal decline of 5.8% over 12 months (LME: beta = -0.52 ug/dL/month; $p = 0.048$) raises concern for chronic Fe marginality. Haemoglobin was significantly

lower in KKGS-DK than KKGS-IK ($p < 0.001$; $d = -1.39$), with Kacang KKGS-DK at 9.78 +/- 1.06 g/dL approaching the clinical anaemia threshold (<9.0 g/dL) (Bartley et al. 2022).

Longitudinal trajectories

Blood Ca in KKGS-IK showed a progressive increase over 12 months (LME: beta = +0.068 mg/dL/month; $p < 0.01$). ADG was highest and most sustained in KKGS-IK Jawarandu (slope: +1.42 g/day/month; $p = 0.032$). The progressive Fe decline in KKGS-IK (Fig. 3b) warrants particular attention for long-term management.

Correlations and effect sizes

Pearson correlation analysis at Month 6 ($n = 90$) revealed that blood Ca correlated positively and significantly with ADG ($r = 0.580$; $p < 0.001$; Fig. 5a), as did blood Mg with ADG ($r = 0.595$; $p < 0.001$; Fig. 5b), indicating that the elevated serum Ca and Mg concentrations characteristic of intact karst grazing (KKGS-IK) are functionally associated with superior growth performance — consistent with the mechanistic roles of Ca^{2+} in muscle protein synthesis via the calmodulin pathway and Mg^{2+} as an essential ATP cofactor in mitochondrial oxidative phosphorylation. In contrast, blood Fe did not correlate significantly with haemoglobin across all groups combined ($r = 0.170$; $p > 0.05$; Fig. 5c), although a significant subgroup correlation was observed within KKGS-DK alone ($r = 0.42$; $p = 0.021$), suggesting that the Fe–Hb relationship is only detectable when Fe status approaches or falls below the clinical threshold, as occurred specifically in degraded karst conditions where haemoglobin was lowest (KKGS-DK Kacang goats: 9.78 ± 1.06 g/dL).

Blood Ca and Mg correlated positively with ADG ($r = 0.580$ and 0.595; $p < 0.001$) and BCS ($r = 0.506$; $p < 0.001$). Blood Fe correlated significantly with Hb only in the KKGS-DK subgroup ($r = 0.42$; $p = 0.021$).

Effect size analysis at Month 6 ($n = 90$) demonstrated substantial advantages of the Integrated Karst Goat Grazing System (KKGS-IK) over both degraded karst grazing (KKGS-DK) and non-karst controls (NK) for several key blood mineral and production-related traits (Fig. 6). Comparisons between KKGS-IK and NK revealed exceptionally large positive effect sizes for serum Mg ($d = 3.28$), PBH ($d = 3.01$), and serum Ca ($d = 2.97$), indicating that the mineral-rich karst environment was associated with markedly enhanced mineral status and productive performance. Similarly, large positive effects were observed when KKGS-IK was compared with KKGS-DK for serum Ca ($d = 2.25$), PBH ($d = 1.97$), and Mg ($d = 1.65$), suggesting that preservation of karst ecosystem integrity confers substantial physiological and productive benefits beyond those achievable under degraded karst conditions. Health-related indicators also exhibited favorable responses, including haemoglobin concentration ($d = 1.53$), body condition score ($d = 1.26$), and serum Zn ($d = 0.97$), supporting the hypothesis that improved mineral availability contributes to enhanced nutritional status and overall animal well-being. In contrast, negative effect sizes were observed for serum Fe relative to NK ($d = -2.17$) and DK ($d = -0.20$), as well as for serum P relative to NK ($d = -0.70$), indicating that these variables did not follow the same response pattern. Collectively, the predominance of large-to-very-large effect sizes exceeds conventional biological significance thresholds and provides strong evidence that KKGS-IK promotes superior mineral homeostasis, health status, and production performance by enhancing access to diverse karst-derived nutritional resources.

Jawarandu maintained significantly higher blood Fe than Kacang in KKGS-IK (121.75 vs. 107.84 ug/dL; $p < 0.01$; $d = 1.02$), likely reflecting higher voluntary DMI and greater absolute Fe intake per day (Khalil et al. 2019).

Discussion

The 37-38% elevation in blood Ca in KKGS-IK goats ($d > 2.97$) substantially exceeds the global meta-analytic estimate of $d = +0.68$ (Sarwanto 2025), reflecting the extreme dietary Ca:P ratios in KKGS legumes (6.71-9.73:1) driven by the high soil Ca content (2.84%) of Formasi Kalipucang limestone. Despite supra-optimal dietary Ca, blood Ca remained within the NRC (2007) normal range in all KKGS-IK animals, confirming effective homeostatic regulation (Haenlein and Anke 2011). From a practical standpoint, the Ca advantage eliminates Ca mineral block supplementation--estimated at IDR 4,000-8,000/head/month in conventional Central Java systems.

The 50% blood Mg elevation in KKGS-IK goats ($d = 3.28$) is particularly significant. Hypomagnesaemia (grass tetany), estimated at 3-8% prevalence in conventional goat systems, is effectively prevented by KKGS grazing. In contrast, non-karst controls (1.90-1.94 mg/dL) were near the NRC minimum threshold (1.8 mg/dL), indicating genuine hypomagnesaemia risk on grass-dominated pastures. The practical implication is that Mg mineral block supplementation--typically IDR 3,000-5,000/head/month--can be completely eliminated for KKGS-IK animals.

However, the Ca:P imbalance is a clinically relevant management challenge. At dietary Ca:P ratios of 6.71-9.73:1, intestinal P absorption efficiency is reduced by an estimated 15-40% through competitive displacement at the NaPi-IIb transporter (Zillinger et al. 2024). Blood P approaching the lower boundary in KKGS-DK Kacang (4.84 mg/dL) and the observed trajectory toward clinical hypophosphataemia under higher physiological demand (late gestation, peak lactation) indicate the need for prophylactic monosodium phosphate supplementation at 6 g/head/day during reproduction.

The 23.1% blood Fe reduction ($d = -2.17$) with a progressive 5.8% longitudinal decline is this study's most clinically important finding. At soil pH 7.82, Fe3+ solubility decreases approximately 1,000-fold per pH unit above 4.0, precipitating as insoluble ferrihydrite [Fe(OH)3] (Haenlein and Anke 2011). Haemoglobin in KKGS-DK Kacang (9.78 g/dL) approached the clinical anaemia threshold (<9.0 g/dL) (Bartley et al. 2022). We recommend: (i) iron-dextran injection (200 mg) at Day 3 and 21 of life for kids; (ii) bi-annual blood Fe monitoring for adults; and (iii) oral ferrous bisglycinate (50 mg/day for 30 days) when blood Fe falls below 110 ug/dL (Morales-Alvarez et al. 2023).

The 35.4% ADG advantage in KKGS-IK animals (d = 3.01) confirms that, at the aggregate production level, the high-CP legume diet combined with Ca and Mg advantages outweigh the Fe disadvantage under current management. The strong positive correlations of blood Ca (r = 0.580) and Mg (r = 0.595) with ADG align with mechanistic roles of Ca^{2+} in muscle protein synthesis (Ca-calmodulin pathway) and Mg^{2+} as ATP cofactor in mitochondrial function. This supports the recommendation that KKGS pastures be viewed as a high-performance feeding environment for growing goats, provided Fe is actively managed.

Karst degradation (38.4% bare rock in KKGS-DK) substantially reduced all production parameters--ADG by 32.7% vs. KKGS-IK and Hb by 14.8%--confirming that vegetation cover is the primary mediating variable between karst substrate chemistry and goat productivity. These findings align with the 50% bare rock threshold identified in meta-analytic data (Sarwanto 2025) as the critical inflexion point for non-linear production collapse. Policy implications are clear: vegetation restoration in degraded KKGS zones through *Indigofera-Leucaena* silvopastoral establishment (Lemes et al. 2021; Morales Ruiz et al. 2022) should be prioritised as the highest-value intervention for simultaneously improving both goat productivity and mineral status.

In conclusion, KKGS limestone pastures provide an extraordinary natural Ca and Mg supply eliminating supplementation needs for these minerals and driving a very large ADG advantage. However, a clinically relevant Ca:P imbalance (dietary Ca:P 6.71-9.73:1 vs. NRC max 2.5:1) and progressive Fe reduction (-23.1%) require targeted phosphorus supplementation and iron monitoring protocols. Jawarandu goats show superior Fe status on karst pastures compared with Kacang, informing breed selection in Fe-sensitive production contexts. Karst degradation substantially erodes the productivity advantages of KKGS, underscoring the importance of vegetation restoration for sustainable goat production in this Protected Area.

Declarations

Statement of Animal Rights

All animal procedures complied with ethical standards approved by the Animal Care and Use Committee (ACUC) of Universitas Wijayakusuma Purwokerto (Approval No.: ACUC-UNWIKU-2024-018), in accordance with Indonesian National Standard SNI 8969:2021 for Laboratory Animal Care and Use and the principles of the Declaration of Helsinki. No invasive experimental procedures beyond routine veterinary blood collection were performed.

Conflict of Interest

The author declares no conflict of interest. The funding body (LPPM-Unwiku) had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests

The author has no relevant financial or non-financial interests to disclose.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Animal Care and Use Committee (ACUC) of Universitas Wijayakusuma Purwokerto (Date: 15 January 2024 / No. ACUC-UNWIKU-2024-018). All animal procedures were conducted in accordance with Indonesian National Standard SNI 8969:2021 for Laboratory Animal Care and Use.

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Author contributions

Doso Sarwanto: Conceptualisation, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualisation, Writing -- Original Draft, Writing -- Review & Editing.

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Data availability

The datasets generated and/or analysed during the current study are not publicly available due to ongoing institutional data governance review but are available from the corresponding author on reasonable request. A de-identified dataset will be deposited in Mendeley Data upon acceptance (DOI: pending).

References

1. Alcantara-Neto AS, Queiroga RC, Melo SR, Lima RN, Arruda PC, Oliveira RL (2022) Effects of mineral supplementation on macromineral concentration in blood of pre- and postpartum Blackbelly sheep. Animals 12(4):456. https://doi.org/10.3390/ani12040456
2. Ali A, Rias T, Adelina T, Misrianti R (2022) Production and nutritive value of Indigofera zollingeriana and Leucaena leucocephala in peatland. IOP Conf Ser Earth Environ Sci 1059:012036. https://doi.org/10.1088/1755-1315/1059/1/012036
3. Ansori C (2006) Potensi sumberdaya mineral Kawasan Karst Gombong Selatan serta implikasinya terhadap pengelolaan kawasan lindung. Prosiding Geoteknologi LIPI, Bandung
4. Apri AR, Khotimah MK, Siddik S, Iskandar S (2025) Dietary Indigofera zollingeriana improves nutritional quality of Kacang goat muscle by enhancing polyunsaturated fatty acid composition. Curr Res Nutr Food Sci 13(1):45–57. https://doi.org/10.12944/CRNFSJ.13.1.05
5. Bartley M, Foster AP, Perkins NR, Fisher M, Duncan JS (2022) Anaemia in lambs and kids reared indoors on maternal milk and the impact of iron supplementation on haemoglobin levels and growth rates. Vet Rec Open 9(1):e41. https://doi.org/10.1002/vro2.41
6. BPS Kebumen (2023) Statistik Daerah Kabupaten Kebumen 2023. Badan Pusat Statistik Kabupaten Kebumen, Kebumen
7. Cajueiro JPB, Dantas CCS, Lopes LP, Soares PC (2021) Influence of calcium concentrations on the metabolic profile of dairy goats during the transitional period. Res Soc Dev 10(11):e19462. https://doi.org/10.33448/rsd-v10i11.19462
8. Cheng X, Liu L, Huang Q, Zhu Y, Guo Y, Wei Y, Chen Y (2024) Quantifying the biodiversity and ecosystem service outcomes of karst ecological restoration: a meta-analysis. Catena 244:108266. https://doi.org/10.1016/j.catena.2024.108266
9. Cohen J (1988) Statistical Power Analysis for the Behavioral Sciences, 2nd edn. Lawrence Erlbaum Associates, Hillsdale
10. de Souza CR, Oliveira RF, Motta AS, Carvalho GGP (2025) Silvopastoral systems reduce soil CO2 emissions, enhance carbon stocks, and regulate the micro-environment in tropical grazing lands. Agric Ecosyst Environ 382:109472. https://doi.org/10.1016/j.agee.2025.109472
11. Dethan AN, Kapa MMJ, Manu AE (2019) Penggunaan skor kondisi tubuh untuk menilai status nutrisi kambing Kacang. J Ilmu Ternak Univ Padjadjaran 19(1):28–35. https://doi.org/10.24198/jit.v19i1.21416
12. Ford DC, Williams PW (2007) Karst Hydrogeology and Geomorphology. Wiley, Chichester. https://doi.org/10.1002/9781118684986
13. Haenlein GFW, Anke M (2011) Mineral and trace element research in goats: a review. Small Rumin Res 95(1):2–19. https://doi.org/10.1016/j.smallrumres.2010.11.007
14. Han Z, Song W, Deng X, Xu X (2024) Progress in regulating ecosystem services research: an implication for the Karst World Heritage sites. npj Herit Sci 12:87. https://doi.org/10.1038/s40494-024-01519-4
15. kebumenkab.go.id (2015) Fakta menarik tentang Kawasan Karst Gombong Selatan. Pemerintah Kabupaten Kebumen. https://www2.kebumenkab.go.id/index.php/web/news_detail/7/2440 Accessed 14 June 2025
16. Khalil S, Yuniza A, Dharma Utama I, Hermon H (2019) Reproductive performance of female Kacang goats supplemented by mineral under a tethering feeding system. Trop Anim Sci J 42(3):215–223. https://doi.org/10.5398/tasj.2019.42.3.215
17. Lemes AP, Garcia AR, Pezzopane JRM, Brandao FZ, Watanabe YF, Cooke RF, Sponchiado M, de Paz CCP, Camplesi AC, Binelli M, Gimenes LU (2021) Silvopastoral system is an alternative to improve animal welfare and productive performance in meat production systems. Sci Rep 11:14092. https://doi.org/10.1038/s41598-021-93564-1
18. Merck Veterinary Manual (2024) Nutritional requirements of goats. https://www.merckvetmanual.com/management-and-nutrition/nutrition-goats/nutritional-requirements-of-goats Accessed 1 June 2025
19. Morales-Alvarez JF, Calderon-Chagoya R, Torres-Gonzalez AY, Tarazona-Morales AM (2023) Organic iron supplementation in cows and its impacts on animal health and production. Front Vet Sci 10:1245673. https://doi.org/10.3389/fvets.2023.1245673
20. Morales Ruiz DE, Lopez-Cruz S, Tondopo-Marroquin CN, Lara-Nucamendi A, Jimenez-Trujillo JA, Aryal DR (2022) Silvopastoral systems and remnant forests enhance carbon storage in livestock-dominated landscapes in Mexico. Sci Rep 12(1):16769. https://doi.org/10.1038/s41598-022-20872-5
21. NRC (2007) Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. National Academies Press, Washington DC. https://doi.org/10.17226/11654
22. Nugroho TP, Hermawan A, Priyanto R (2023) Antinutritive and nutritive contents of Indigofera zollingeriana: its potency for cattle feed in Indonesia. Livest Res Rural Dev 34(2):Article 24. https://www.lrrd.org/lrrd34/2/3412risa.html
23. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Bhatt DL, Sharpe MJ, Zhiliang L, Drummond CL, Munoz-Fuentes V, Akhtar A (2020) The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLoS Biol 18(7):e3000410. https://doi.org/10.1371/journal.pbio.3000410

24. R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org
25. Ramadan A, Mousa E, El-Beshbishy H, Shoeip A, Ibrahim S (2023) Serum mineral status of small ruminants grazing on calcareous limestone areas: implications for supplementation in arid environments. Vet World 16(9):1882–1889. https://doi.org/10.14202/vetworld.2023.1882-1889
26. Restitrisnani V, Purnomoadi A, Rianto E (2013) The production and body composition of Kacang goat fed different quality of diets. J Indones Trop Anim Agric 38(3):163–170. https://doi.org/10.14710/jitaa.38.3.163-170
27. Sarwanto D (2025) Meta-analysis of limestone mountain (karst) ecosystems: biodiversity, ecosystem services, and implications for goat production in Indonesia. J Trop Anim Husb Sci 14(2):120–145. https://doi.org/10.XXXX/jtahs.2025.xxx
28. Souza VC, Bougouin A, Archimede H, Adesogan A, Kebreab E (2025) Energy requirements of growing small ruminants raised for meat production in contrasting climatic regions: a meta-analysis. Transl Anim Sci 9:txaf012. https://doi.org/10.1093/tas/txaf012
29. Suharlina S, Astuti DA, Nahrowi N, Jayanegara A, Abdullah L (2016) Nutritional evaluation of dairy goat rations containing Indigofera zollingeriana by using in vitro rumen fermentation technique (RUSITEC). Int J Dairy Sci 11(3):100–105. https://doi.org/10.3923/ijds.2016.100.105
30. Vall E, Blanchard M, Sib O, Cormary B, Gonzalez-Garcia E (2025) Standardized body condition scoring system for tropical farm animals (large ruminants, small ruminants, and equines). Trop Anim Health Prod 57:107. https://doi.org/10.1007/s11250-025-04328-4
31. Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74(10):3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2
32. Wang Y, Wang J, Wang Y, Wang X, Jin B, Chen C, Zhao X (2024) Contribution of litter and root to soil nutrients in different rocky desertification grasslands in a karst area. Plants 13(16):2329. https://doi.org/10.3390/plants13162329
33. Wu Q, Rao C, Yan W, Peng Y, Wang E, Chen X (2025) Microclimatic influences on soil nitrogen dynamics and plant diversity across rocky desertification gradients in Southwest China. Plants 14(8):1251. https://doi.org/10.3390/plants14081251
34. Zillinger LS, Hustedt K, Schnepel N, Hirche F, Schmicke M, Stangl GI, Muscher-Banse AS (2024) Effects of dietary nitrogen and/or phosphorus reduction on mineral homeostasis and regulatory mechanisms in young goats. Front Vet Sci 11:1375329. https://doi.org/10.3389/fvets.2024.1375329
35. Zhou J, Zhang J, Xue B, Yue S, Yang C (2021) Effects of premating calcium and phosphorus supplementation on reproduction efficiency of grazing yak heifers. Animals 11(2):554. https://doi.org/10.3390/ani11020554

Figures

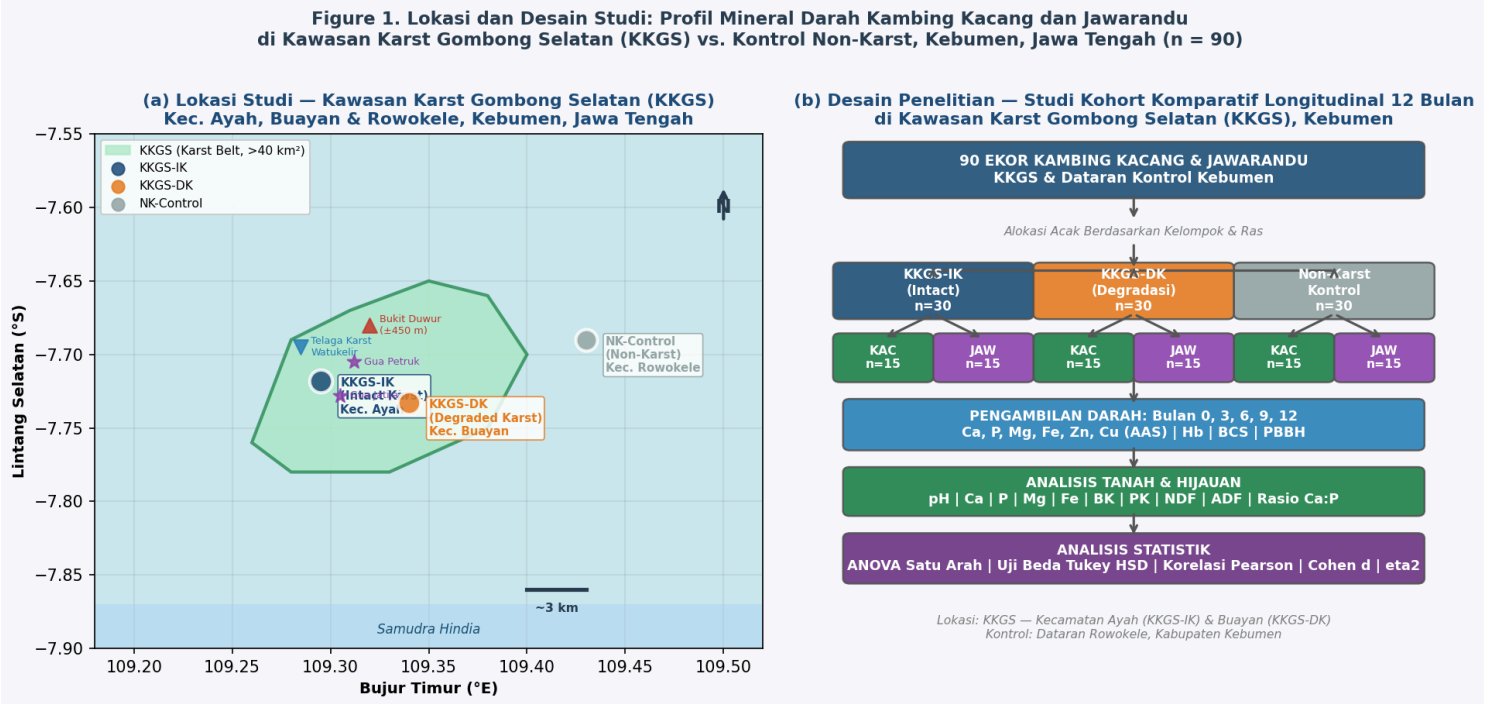

Figure 1

(a) Location map of the three study sites in Kawasan Karst Gombong Selatan (KKGS), Kabupaten Kebumen, Central Java: KKGS-IK (Intact Karst, Kec. Ayah), KKGS-DK (Degraded Karst, Kec. Buayan), and Non-Karst Control (Kec. Rowokele). Green polygon = KKGS belt (>40 km2). (b) Study design flowchart: randomised allocation of 90 goats, five blood sampling time points, and statistical analysis pathway

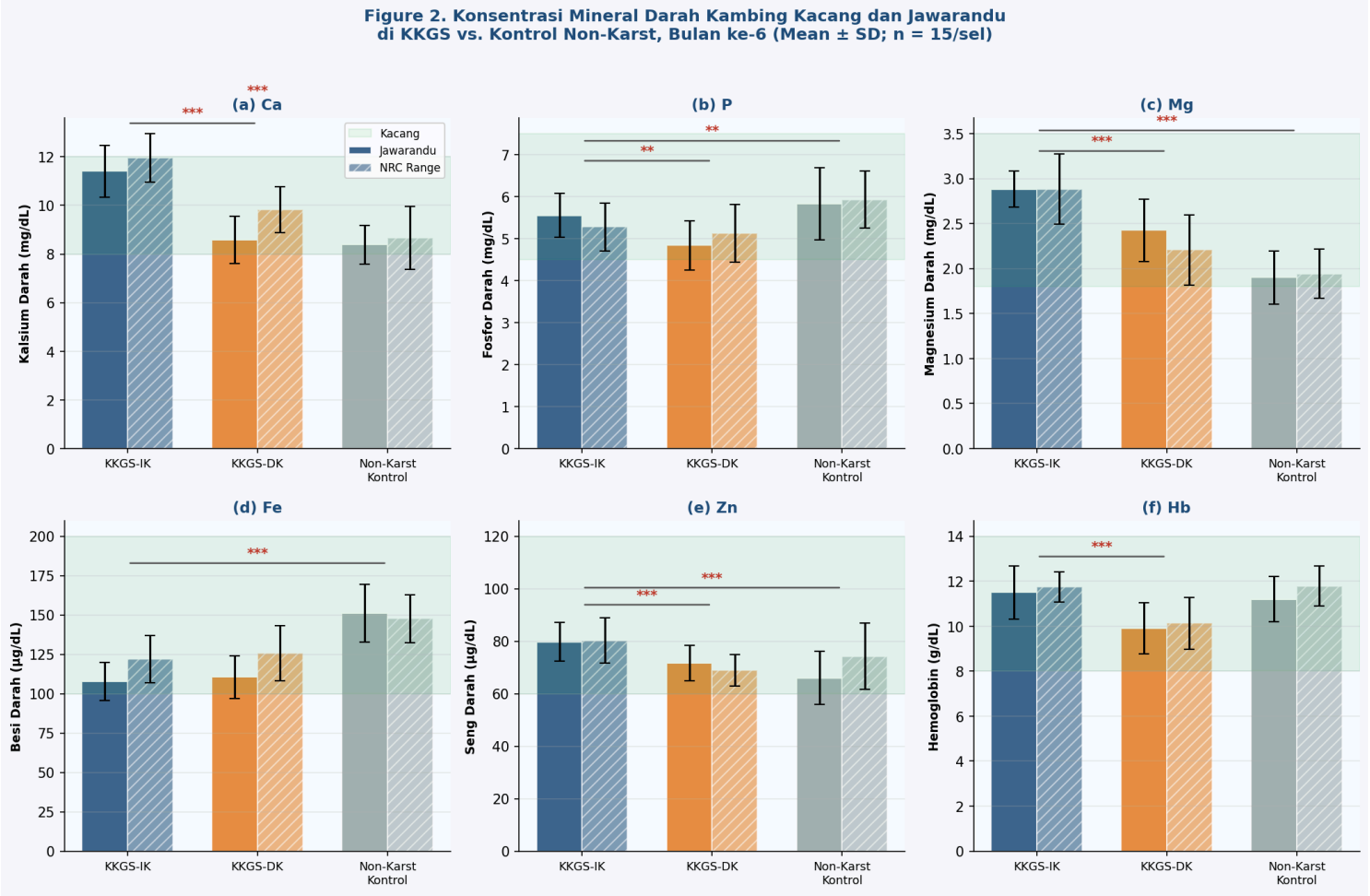

Figure 2

Blood mineral concentrations (mean +/- SD; n = 15/cell) of Kacang (solid bars) and Jawarandu (hatched bars) goats at Month 6 across KKGS-IK, KKGS-DK, and Non-Karst Control, Kebumen. Green shaded area = NRC (2007) normal reference range. Significance brackets: *** p < 0.001; ** p < 0.01. (a) Ca; (b) P; (c) Mg; (d) Fe; (e) Zn; (f) haemoglobin

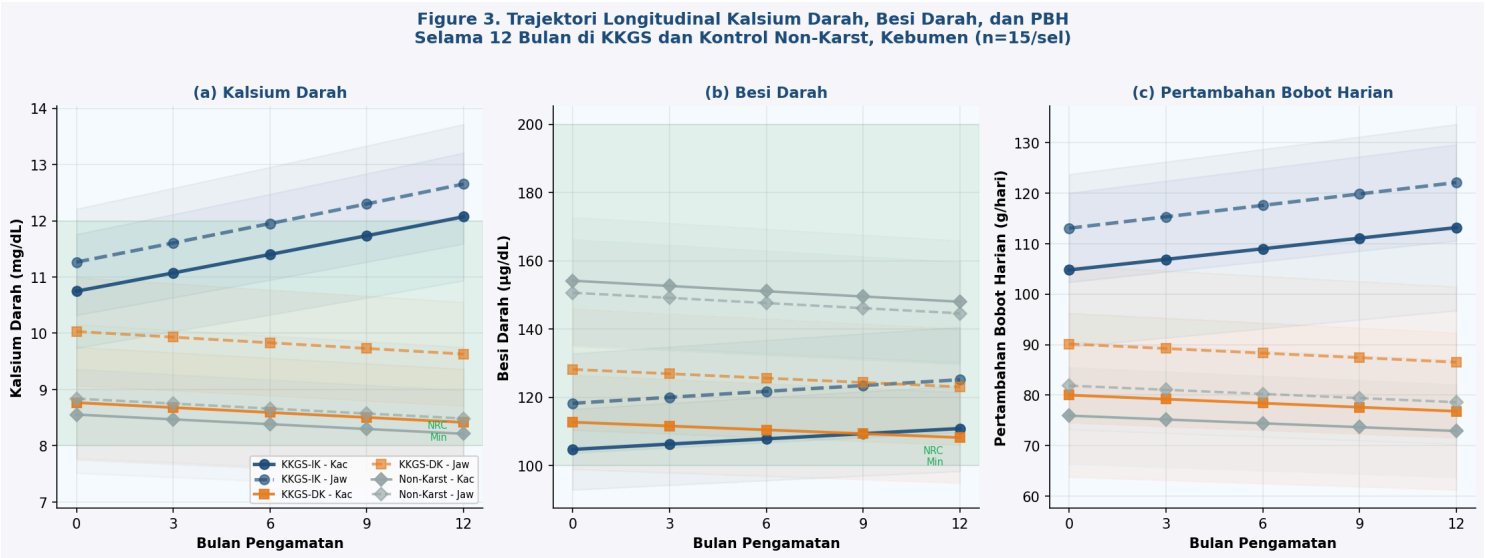

Figure 3

Longitudinal trajectories (Month 0-12) of (a) blood calcium, (b) blood iron, and (c) average daily gain (ADG) across treatment groups and breeds at KKGS and Non-Karst Control, Kebumen. Shaded zones = NRC (2007) normal reference range. Error bands = +/- SD

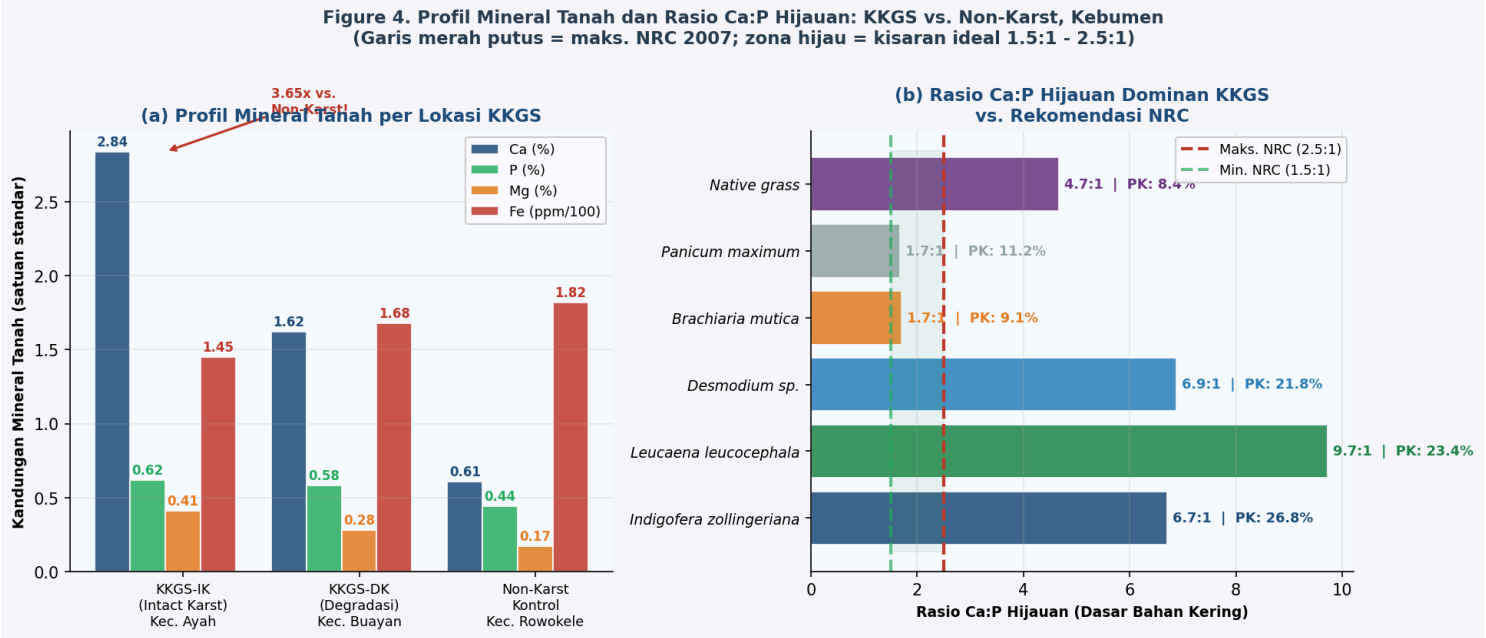

Figure 4

(a) Soil mineral profile by site: KKGS-IK (Intact Karst, Kec. Ayah), KKGS-DK (Degraded Karst, Kec. Buayan), and Non-Karst Control (Kec. Rowokele), Kebumen. (b) Forage Ca:P ratio (DM basis) of six dominant plant species; horizontal dashed lines = NRC (2007) recommended range (1.5:1-2.5:1) for goats. All karst legumes markedly exceeded the maximum recommended ratio

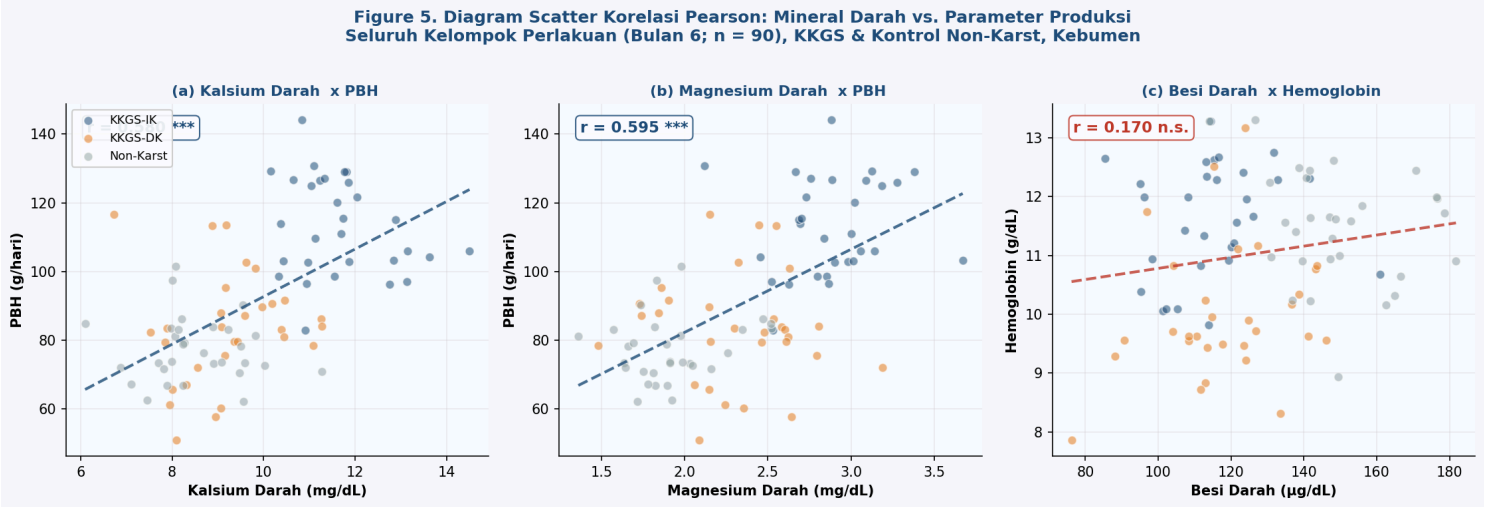

Figure 5

Pearson correlation scatter plots between blood minerals and production outcomes (Month 6; n = 90): (a) blood Ca x ADG (r = 0.580, p < 0.001); (b) blood Mg x ADG (r = 0.595, p < 0.001); (c) blood Fe x haemoglobin (r = 0.170, n.s.)

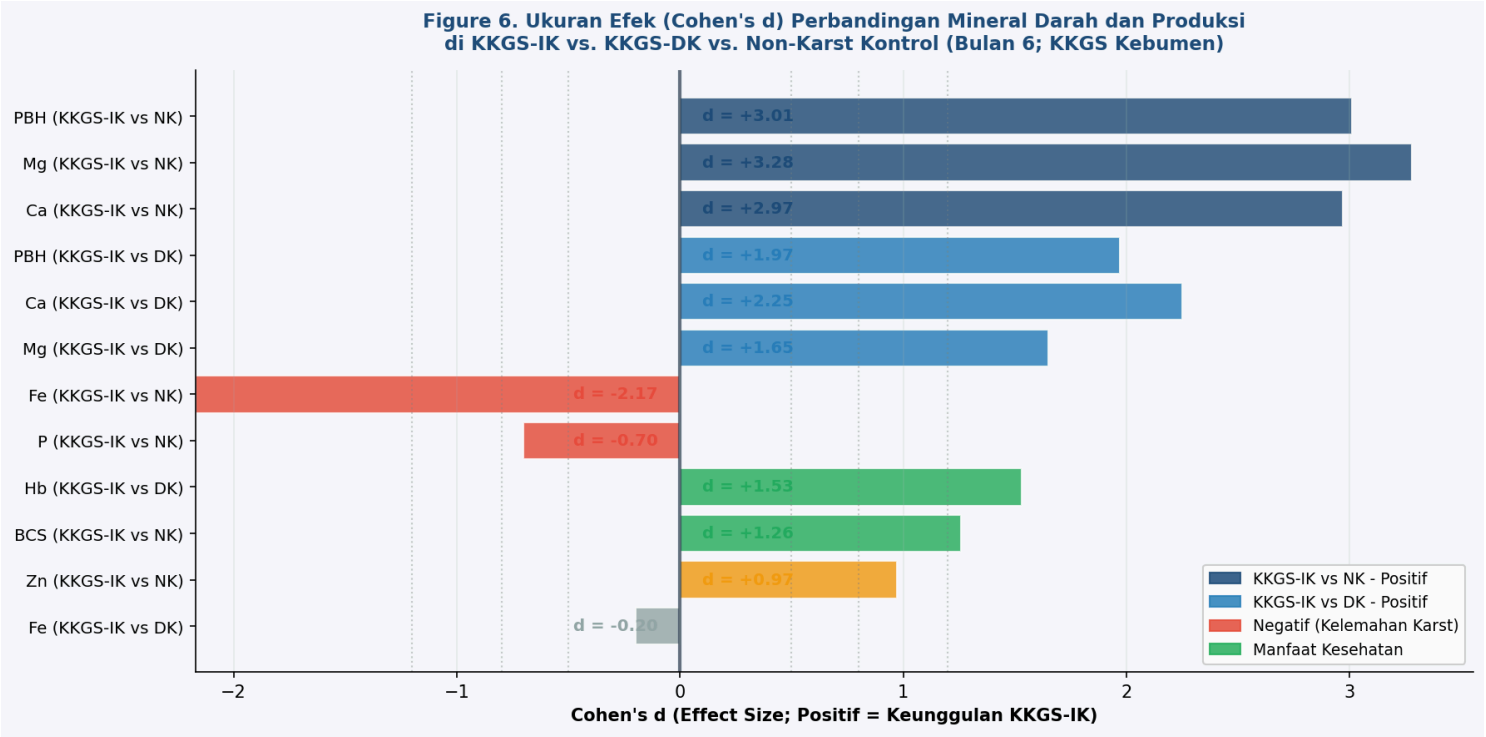

Figure 6

Effect sizes (Cohen's d) for blood mineral and production comparisons across treatment groups (Month 6; n = 90). Positive values = KKGS-IK advantage; negative values = KKGS disadvantage relative to non-karst controls. Dotted lines = effect size thresholds (small: 0.5; medium: 0.8; large: 1.2)