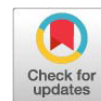


Research Article



Efficacy of Herbal Mineral Supplements in Different Feed Forms on Foot and Mouth Disease (FMD) Immunity in Cattle

ASHARIAH HAPILA, DILLA MAREISTIA FASSAH, HASNA RABILA, ASEP SUDARMAN*

Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia.

Abstract | Foot and Mouth Disease (FMD) is a highly contagious disease affecting hoofed animals, causing significant economic losses worldwide. This study evaluated the efficacy of herbal mineral supplementation containing turmeric, ginger, zinc glycine, and nano selenium on nutrient intake, immune function, and overall performance in FMD vaccinated cattle. The supplements were provided in three feed forms: Mash, pellet, and herbal mineral block (HMB). Twelve cattle were assigned to three feed form treatments, with four animals per treatment serving as individual replicates. The experiment was conducted over six weeks period. Parameters measured included feed intake, nutrient digestibility, blood metabolites, haematology, liver enzymes, and FMD specific IgG titers. Results showed that mash feed significantly increased ($p < 0.05$) antibody titers, feed efficiency and average daily gain (ADG) compared to pellets and HMB. Both mash and pellet forms reduced alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, suggesting improved liver health. These findings indicate that mash feed is the most effective form for delivering herbal-mineral supplementation to enhance FMD immunity and cattle performance.

Keywords | Cattle nutrition, Feed forms, Foot and mouth disease, Herbal mineral supplementation, Immune response

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***Correspondence** | Asep Sudarman, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia.;

Email: asudarman@apps.ipb.ac.id

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INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious viral infection that primarily affects cloven hoofed livestock, including cattle, sheep, goats, buffalo, and pigs. It is prevalent in Asian countries (Ismail *et al.*, 2025; Kabir *et al.*, 2024, 2025). In Indonesia, recurrent FMD outbreaks result in infection rates surpassing 90% in cattle populations, causing substantial economic losses through decreased productivity, trade restrictions, and elevated morbidity rates (Nurul *et al.*, 2022). While vaccination remains the cornerstone of FMD control strategies, the protective immunity conferred is often short lived, typically lasting only 4–6 months, thereby necessitating adjunct strategies

to improve vaccine efficacy and longevity (Stenfeldt and Arzt, 2020).

Recent research has highlighted the pivotal role of nutritional immunomodulation in enhancing host immune responses and supporting disease resilience in livestock. Trace elements such as zinc and selenium, particularly in bioavailable forms like zinc glycine and nano selenium, have demonstrated the capacity to stimulate lymphocyte proliferation and augment antioxidant defenses (Prasad and Bao, 2019; Ma *et al.*, 2013; Wijayanti *et al.*, 2023). Concurrently, phytochemical compounds such as curcumin and gingerol derived from turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*), respectively exhibit

pronounced anti inflammatory and immunomodulatory effects, contributing to improved vaccine responsiveness (Abdelnour *et al.*, 2021).

In practical ruminant nutrition, urea molasses mineral blocks (UMBs) serve as a widely adopted vehicle for controlled nutrient delivery, while pelleted feed formulations are increasingly favored over mash due to their improved handling, nutrient uniformity, and reduced wastage (Zhao *et al.*, 2022; Mohd-Nor *et al.*, 2020; Ebrahimi, 2020). However, the comparative efficacy of these feed delivery systems particularly mash, pellet, and herbal mineral block (HMB) forms in modulating immune responses in FMD vaccinated beef cattle remains insufficiently investigated. Previous studies on feed form have mainly focused on growth performance rather than immune parameters (Zaefarian *et al.*, 2016), leaving a significant gap in understanding their potential immunomodulatory effects.

This study aims to evaluate the influence of different feed delivery forms of herbal mineral supplements on nutrient intake, immune function, and overall performance in FMD vaccinated cattle. The findings are expected to support the development of optimized nutritional strategies that enhance the effectiveness of vaccination protocols and strengthen herd-level immunity.

MATERIALS AND METHODS

ANIMALS AND DIETS

Twelve adult Bali cattle (n = 9) and Madura cattle (n = 3), weighing 158–250 kg were housed individually and fed experimental feeds for 42 days with two week adaptation period preceded the trial. The animals were randomly assigned to three feed-form treatments (with four animals per treatment): mash, pellet, and HMB, with four replicates per group. The feed was formulated to meet the nutrient requirements of beef cattle with a forage to concentrate ratio of 53:47. The ration was supplemented with 2% turmeric powder, 1% ginger powder, 300 mg head⁻¹ zinc (as zinc glycine), and 1 mg head⁻¹ selenium (as nano selenium) (Kesler and Abuelo, 2024; Franco *et al.*, 2022; Deepigaa *et al.*, 2021; Mandal *et al.*, 2007).

All treatment groups (T1, T2, and T3) received the same composition and dosage of herbal and mineral additives; the only difference among treatments was the physical form in which these additives were delivered. Specific dietary treatments included:

- T1: Ration + herbal + zinc + selenium in mash form.
- T2: Ration + herbal + zinc + selenium in pellet form.
- T3: Ration in mash form + herbal + zinc + selenium as suspended mineral blocks.

FEED PREPARATION

The ration was formulated to meet the nutritional requirements for beef cattle, with a total digestible nutrient (TDN) level exceeding 60% (NRC, 2000). The diet consisted of a mixture of green fodder and commercial concentrates, maintaining a forage to concentrate ratio of 53:47. The feed components included copra cake, cassava pulp, lime, salt, corn gluten feed (CGF), molasses, dicalcium phosphate (DCP), coffee husks, palm kernel cake, pollard, and a premix. Herbal additives were incorporated at 3% of the total dry matter intake, with zinc provided at 75 ppm per head per day and selenium at 0.25 ppm per head per day. The feed was then processed into the respective forms (mash, pellets, and herbal mineral blocks) according to the experimental treatment design. Mash and pellet feeds were processed using standard methods, while HMB was produced with molasses as a binder and hung for cattle to lick. The nutrient composition of the feed was verified to ensure consistency across forms. Total Digestible Nutrients (TDN) were calculated using the following formulas, as described by Wardeh (1981):

For forage: $TDN = -21.7656 + (1.4284 \times \%CP) + (1.0277 \times \%NFE) + (0.4867 \times \%EE)$
For concentrate: $TDN = 40.2625 - (0.1379 \times \%CF) + (1.1903 \times \%EE) + (0.4228 \times \%NFE) + (0.1969 \times \%CP)$

The nutrient composition of the feedstuffs is presented in Table 1.

Table 1: Nutrient composition of experimental feeds (%).

Nutrient con- tent	Elephant grass	Rice straw	Commercial concentrate	Mash	Pellet
Dry matter	89.87	89.36	88.63	87.3	87.62
Crude protein	7.21	8.49	13.59	12.91	12.34
Ether extract	3.19	3.71	3.16	2.33	2.48
Crude fibre	28.31	34.05	13.84	10.7	12.15
Ash	11.52	17.71	5.17	3.51	4.82
Nitrogen free extract	49.77	36.04	64.24	70.55	68.21
TDN*	57.39	47.5	71.95	73.93	72.81

* TDN: Total digestible nutrients.

EXPERIMENTAL DESIGN

The study followed a randomized block design with three treatments and four replicates. Measurements included feed intake, nutrient digestibility, blood metabolites, liver enzyme activity, haematology, and FMD immunoglobulin G (IgG) titers. These parameters were also measured on day 0 (prior to the supplementation) and used as baseline controls for each animal.

CATTLE PERFORMANCE

Cattle were weighed before and after the trial to calculate

average daily gain (ADG). Feed intake was monitored daily by offering feed twice daily according to the schedule: concentrate at 08:00 AM and 02:00 PM, and forage at 10:00 AM and 04:00 PM. Refusals were collected and weighed the following morning, and daily feed intake was calculated as the difference between the total amount offered and the amount refused.

The herbal additives (turmeric and ginger powders) and mineral supplements (zinc glycine and nano selenium) were premixed thoroughly with the basal diet to ensure homogeneous distribution. Actual intake of these additives was then calculated indirectly by multiplying the proportion of each additive in the diet by the daily feed intake recorded for each animal. For the HMB treatment, daily intake was determined by measuring the difference in block weight before and after the experimental period, divided by the number of days, and then multiplied by the fixed dose of herbal and mineral additives contained in the HMB.

NUTRIENT DIGESTIBILITY

Digestibility was assessed over seven days using the total faecal collection method, conducted at the end of the study (Aling *et al.*, 2020). Fresh faeces were collected, air-dried for 24 hours, and then oven-dried at 60°C for another 24 hours. The composite feed samples were analyzed for nutrient composition, including dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen-free extract (NFE), following the method described by Sastradipradja and Hartini (1989).

HAEMATOLOGY AND BLOOD METABOLITE MEASUREMENTS

Blood sampling for metabolite and haematology was conducted twice, on day 0 and day 42 at 06.00 AM before feeding. Approximately 10 ml of blood was drawn from the jugular vein using a disposable syringe and transferred into a 6 ml Ethylene Diamine Tetraacetic Acid vacuum tube and a 3 ml heparin vacuum tube. Red and white blood cell counts were performed using the counting chamber method (Sastradipradja and Hartini, 1989). Haematocrit levels were measured via centrifugation (Majid *et al.*, 2023), while white blood cell differentiation was assessed using Giemsa staining (Rinny and Sherly, 2018). Haemoglobin levels were determined using the Sahli method (Sastradipradja and Hartini, 1989). The red blood cell index and inflammation ratio were calculated using the formulas provided in the references (Beck, 2009).

Blood metabolite analyses were performed using a Microlab 300 instrument based on enzymatic reactions with the kit method. The analyses used specific kits for glucose (REF 10260, Human, Germany), triglycerides (REF 10724,

Human, Germany), and protein (REF 157004, Human, Germany).

LIVER ENZYMES AND IgG TITER MEASUREMENT

Blood sampling for liver enzymes was conducted twice on day 0 and 42 along with antibody testing were collected on day 28 and 42 in the morning before feeding, via the jugular vein using vacutainer tubes. Blood metabolite analysis was conducted using enzymatic colorimetric methods via commercial diagnostic kits. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which serve as indicators of liver function, were measured using kits (REF 77169 for AST/GOT and REF 77170 for ALT/GPT; Arkray, Japan). Briefly, 100 µl of serum sample was added to the sample container and placed on the sample rack according to the kit protocol (Novelia *et al.*, 2016). In order to perform antibody testing, blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum, which was then transferred to Eppendorf tubes and stored at -20°C until analysis. The concentration of FMD IgG antibodies was measured using the Structural Protein Enzyme Linked Immunosorbent Assay (SP ELISA) method with the FMDOC-5P kit (IDvet, France), and results were expressed as percentage inhibition (PI).

STATISTICAL ANALYSIS

Data were analyzed according to a Randomized Complete Block Design (RCBD) with treatment as a fixed effect and block as a random effect. When the ANOVA indicated significant differences ($p < 0.05$), means were separated using Duncan's Multiple Range Test (DMRT). All statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA).

RESULTS

As shown in Table 2, mash feed significantly improved ($p < 0.05$) ADG (22.73 g/kg BW^{0.75}) and feed efficiency (15.49%) compared to pellets and HMB. Similarly, NFE was improved, while CF, CP and EE were reduced in mash feed treatment as compared with pellet and HMB treatments ($p < 0.05$). HMB resulted in the lowest intake of herbal and mineral supplements probably due to its licking-based consumption method. However, no significant differences were observed in dry matter, crude protein, or ether extract digestibility across treatments.

The intake of turmeric, ginger, selenium (Se), and zinc (Zn) was significantly higher ($p < 0.05$) in the mash and pellet feed groups compared to the HMB treatment group (Table 3). However, the effect of different feed forms on nutrient digestibility was not significant (Table 4).

Table 2: Effect of herbal mineral supplementation in different forms on cattle performance.

Performance	Treatment*		
	T1	T2	T3
Feed intake (g kg BW^{-0.75} day⁻¹)			
Total dry matter	150.25 ± 8.28	151.23 ± 8.49	154.08 ± 8.24
Concentrate	72.53 ± 4.79 ^a	73.19 ± 3.52 ^a	75.03 ± 4.19 ^b
Forage	71.85 ± 3.98	70.64 ± 5.43	68.87 ± 5.73
Crude protein	15.71 ± 0.90 ^a	15.66 ± 0.94 ^a	16.83 ± 0.88 ^b
Ether extract	4.32 ± 0.29 ^a	4.53 ± 0.28 ^b	5.14 ± 0.27 ^c
Crude fiber	33.56 ± 1.68 ^a	34.95 ± 2.08 ^b	36.85 ± 1.95 ^c
Nitrogen free extract	80.64 ± 4.68 ^b	79.74 ± 4.34 ^{ab}	79.05 ± 4.12 ^a
Total digestible nutrient	93.34 ± 5.32	93.38 ± 5.13	94.67 ± 5.14
Initial body weight (kg head ⁻¹)	192.12 ± 29.72	195.75 ± 18.9	191.87 ± 40.27
Final Body Weight (kg head ⁻¹)	240.25 ± 31.88	243.87 ± 27.74	238.75 ± 23.68
Average daily gain (g kg ⁻¹ BW ^{0.75} day ⁻¹)	22.73 ± 1.99 ^b	18.20 ± 1.83 ^a	21.46 ± 1.98 ^{ab}
Feed Efficiency (%)	15.49 ± 1.43 ^b	12.74 ± 0.66 ^a	13.28 ± 1.23 ^a

Ab, Different superscripts on the same row indicate significant differences (p < 0.05). * T1: Mash form; T2: Pellet form; T3: HMB (herbal mineral block) form

The significant effect of supplementation over time was showed for mash and pellet treatments (p < 0.05) on blood glucose and triglyceride levels by day 42, indicating better nutrient utilization. Protein levels were also higher in mash

fed cattle (Table 5). Supplementation for mash and pellet forms significantly reduced (p < 0.05) ALT and AST levels, suggesting improved liver health. Mash groups indicated significant (p < 0.05) increases in IgG titers by day 42, demonstrating enhanced adaptive immunity (Table 6). Notably, mash feed consistently outperformed other forms in promoting immune response. Mash feed improved Haemoglobin and haematocrit levels, while HMB showed elevated inflammation ratios, indicating stress due to insufficient herbal and mineral intake (Table 7).

Table 3: Effect of herbal-mineral supplementation in different forms on the intake of herbs and minerals.

Supplementation	Treatment*		
	T1	T2	T3
Turmeric (g head ⁻¹ day ⁻¹)	78.85±1.54 ^b	79.31±1.08 ^b	19.51±0.34 ^a
Ginger (g head ⁻¹ day ⁻¹)	39.42±0.77 ^b	39.65±0.54 ^b	9.76±0.17 ^a
Zn (mg head ⁻¹ day ⁻¹)	296.04± 5.78 ^b	297.43±4.06 ^b	73.25±1.29 ^a
Se (mg head ⁻¹ day ⁻¹)	0.98±0.019 ^b	0.99±0.013 ^b	0.24±0.004 ^a

Ab, Different superscripts on the same row indicate significant differences (p < 0.05). * T1: Mash form; T2: Pellet form; T3: HMB (herbal mineral block) form

Table 4: Effect of herbal-mineral supplementation in different forms on nutrient digestibility (%).

Nutrient	Treatment*		
	T1	T2	T3
Dry matter	86.99 ± 1.02	87.73± 1.15	86.44 ± 1.98
Crude protein	84.19 ± 1.33	84.77 ± 1.29	84.60 ± 2.00
Ether extract	94.23 ± 2.26	93.85 ± 0.96	94.68 ± 1.23
Crude fibre	82.41 ± 0.76	84.67 ± 1.42	81.81± 2.95
Nitrogen free extract	90.53 ± 1.08	90.81 ± 1.15	89.74 ± 1.53

* T1: Mash form; T2: Pellet form; T3: HMB (herbal mineral block form)

Table 5: Effect of herbal-mineral supplementation in different forms on blood metabolites.

Blood metabolites	Period	Treatment#			Reference values
		T1	T2	T3	
Glucose (mg dL ⁻¹)	Day-0	48.75 ± 1.69 ^a	61.25 ± 1.32 ^c	53.95 ± 1.67 ^b	43 – 100*
	Day-42	70.70 ± 6.0 ^b	70.83 ± 3.41 ^b	62.72 ± 0.45 ^{ay}	
	Δ	+45.02%	+15.64%	+16.25%	
Triglyceride (mg dL ⁻¹)	Day-0	18.01 ± 0.71 ^c	14.16 ± 0.33 ^b	11.46 ± 1.06 ^a	0 – 14*
	Day-42	27.50 ± 1.27	27.65 ± 2.43	24.77 ± 1.49	
	Δ	+52.69%	+95.26%	+116.14%	
Protein (g dL ⁻¹)	Day-0	7.60 ± 0.46 ^b	7.35 ± 0.63 ^{ab}	7.07 ± 0.37 ^a	5.7 – 8.1**
	Day-42	8.50 ± 0.5	8.02 ± 0.74	7.95 ± 0.62 ^y	
	Δ	+11.84%	+9.11%	+12.44%	

ab) Different superscripts on the same variable row indicate significant differences (p < 0.05). *(Mitruka *et al.*, 1977). ** (Radostis *et al.*, 2007). # T1: Mash form; T2: Pellet form; T3: HMB (herbal mineral block form).

Table 6: Effect of herbal-mineral supplementation in different forms on liver function and foot-and-mouth disease (FMD) immunity.

Parameters	Period	Treatment [#]			Reference Values
		T1	T2	T3	
ALT (IU L ⁻¹)	Day-0	17.25 ± 0.95 ^b	14.50 ± 1.29 ^a	14.25 ± 0.29 ^a	4 – 11 [*]
	Day-42	12.25 ± 0.50 ^a	12.25 ± 1.50 ^a	15.00 ± 1.41 ^b	
	Δ	-28.98%	-15.51%	+7.01%	
AST (IU L ⁻¹)	Day-0	52.50 ± 0.57 ^a	82.00 ± 4.30 ^c	66.75 ± 4.50 ^b	8.5 – 93 ^{**}
	Day-42	34.75 ± 2.36 ^a	47.25 ± 1.89 ^b	49.25 ± 1.89 ^c	
	Δ	-33.81%	-42.37%	-26.21%	
FMD IgG Titer (%)	Day- 28	2.11 ± 0.09 ^c	0.40 ± 0.04 ^a	0.54 ± 0.04 ^b	-
	Day- 42	2.54 ± 0.11 ^c	0.45 ± 0.02 ^a	0.72 ± 0.05 ^b	
	Δ	+20.37%	+12.5%	+33.33%	

ALT = alanine aminotransferase; AST = aspartate aminotransferase. ^{ab}) Different superscripts on the same variable row indicate significant differences (p < 0.05). ^{*}Coles, 1974. ^{**} Mitruka *et al.*, 1977. [#] T1: Mash form; T2: Pellet form; T3: HMB (herbal mineral block).

Table 7: Effect of herbal-mineral supplementation in different forms on haematological parameters.

Haematological parameter	Period	Treatment [#]			Reference values
		T1	T2	T3	
Red blood cells (10 ⁶ uL)	Day-0	4.93 ± 0.45	4.40 ± 0.43	4.52 ± 0.13	4.5 – 6.1 [*]
	Day-42	4.45 ± 0.54	4.36 ± 0.30	4.63 ± 0.40	
	Δ	-9.73%	-0.9%	+1.75%	
Haemoglobin (g dL ⁻¹)	Day-0	8.55 ± 0.75 ^a	8.35 ± 0.10 ^a	9.86 ± 0.92 ^b	7.4 – 9.7 [*]
	Day-42	8.65 ± 0.41 ^a	9.45 ± 0.30 ^a	10.4 ± 0.95 ^b	
	Δ	+1.16%	+11.37%	+5.47%	
Haematocrit (%)	Day-0	26.50 ± 2.51	26.25 ± 1.5	27.25 ± 1.89	27.7 – 30.1 [*]
	Day-42	25.50 ± 1.00 ^a	25.50 ± 0.57 ^a	32.50 ± 1.91 ^b	
	Δ	-3.77%	-2.85%	+15.04%	
MCV (fl)	Day-0	52.76 ± 1.82 ^a	59.97 ± 5.08 ^b	61.41 ± 6.09 ^b	50.5 – 60.1 [*]
	Day-42	57.70 ± 4.92 ^a	58.59 ± 2.89 ^{ab}	70.93 ± 9.37 ^b	
	Δ	+9.36%	-2.3%	+15.5%	
MCH (pg)	Day-0	17.39 ± 1.66	19.13 ± 2.13	18.22 ± 1.90	13.2 – 20.4 [*]
	Day-42	19.67 ± 2.92	21.4 ± 1.85	22.78 ± 2.99	
	Δ	+13.11%	+11.86%	+24.47%	
MCHC (g dL ⁻¹)	Day-0	32.92 ± 2.19	31.87 ± 1.62	29.74 ± 2.38	25.2 – 35 [*]
	Day-42	33.96 ± 2.10 ^{ab}	36.48 ± 1.59 ^b	31.97 ± 1.80 ^a	
	Δ	+3.15%	+14.46%	+7.49%	
White Blood Cells (%)	Day-0	7.47 ± 0.61 ^{bx}	5.68 ± 0.55 ^a	7.95 ± 0.33 ^b	5.1 – 13.3 ^{**}
	Day-42	9.73 ± 0.64 ^{by}	6.50 ± 0.49 ^a	6.91 ± 0.47 ^a	
	Δ	+30.25%	+14.43%	-13.08%	
Lymphocytes (%)	Day-0	59.01 ± 1.08 ^b	53.55 ± 2.38 ^a	54.48 ± 2.05 ^a	45 – 75 ^{***}
	Day-42	61.56 ± 2.62 ^b	57.41 ± 2.71 ^{ab}	52.75 ± 5.07 ^a	
	Δ	+4.32%	+7.21%	-3.17%	
Neutrophils (%)	Day-0	27.40 ± 0.40 ^a	30.56 ± 2.28 ^b	32.06 ± 2.67 ^b	15 – 45 ^{***}
	Day-42	32.26 ± 2.96	33.48 ± 2.84	35.15 ± 3.15	

Table continues on next page.....

Haematological parameter	Period	Treatment#			Reference values
		T1	T2	T3	
	Δ	+17.73%	+9.55%	+9.63%	
Eosinophil (%)	Day-0	9.29 ± 0.67 ^a	10.64 ± 1.1 ^b	9.54 ± 0.42 ^a	2 – 8 ^{***}
	Day-42	6.58 ± 0.62 ^a	8.83 ± 0.61 ^b	8.83 ± 0.47 ^b	
	Δ	-29.17%	-19.58%	-7.44%	
Monocytes (%)	Day-0	3.63 ± 0.36 ^b	2.76 ± 0.326 ^a	3.35 ± 0.27 ^b	2 – 7 ^{***}
	Day-42	3.44 ± 0.14 ^a	3.75 ± 0.13 ^b	3.31 ± 0.06 ^a	
	Δ	-5.23%	+35.86%	-1.19%	
Basophil (%)	Day-0	0.85 ± 0.03	0.95 ± 0.08	0.87 ± 0.07	0 – 2 ^{***}
	Day-42	0.86 ± 0.02	0.90 ± 0.07	0.87 ± 0.04	
	Δ	+1.17%	-5.26%	0	
Inflammation Ratio (%)	Day-0	0.46 ± 0.01 ^a	0.57 ± 0.06 ^b	0.59 ± 0.07 ^b	<1.5 ^{***}
	Day-42	0.52 ± 0.04 ^a	0.58 ± 0.04 ^a	0.72 ± 0.06 ^b	
	Δ	+13.04%	+1.75%	+22.03%	

ab: Different superscripts on the same variable row indicate significant differences. (p < 0.05). xy: Different superscripts in the same variable column (day 0 – day 42) indicate significant differences (p < 0.05). *Siswanto, 2011. **Weiss and Wardrop, 2010. ***Pawitri *et al.*, 2014. # T1: Mash form; T2: Pellet form; T3: HMB (herbal mineral block).

DISCUSSION

Mineral supplementation delivered in the form of molasses blocks has been previously reported to enhance supplement intake, overall feed intake, and nutrient digestibility (Moriel *et al.*, 2019). However, the absence of significant differences in total dry matter intake between treatments in this study suggests comparable palatability across the different feed forms (Table 2). This observation highlights the unique advantages of both mash and pellet rations. Mash feed, composed of smaller particles, shortens retention time in the digestive system, enhancing nutrient absorption (Ebrahimi, 2020). In contrast, pellets provide benefits in terms of feed density and hydration (Raju *et al.*, 2021).

Mash feed resulted in the highest average daily gain (ADG) and feed efficiency compared to the other treatments. This can be attributed to the characteristics of mash, which is more easily consumed and digested by the animals due to its larger surface area and less compact texture (Zaefarian *et al.*, 2016). These properties enhance nutrient availability, thereby supporting more optimal growth. The mash treatment also exhibited superior efficiency values compared to the pellet and HMB groups. One contributing factor is the thermal processing involved in pelleting, which subjects the feed to high pressure and temperature. This process can lead to the degradation of active compounds, such as herbal phytochemicals (curcumin and gingerol), thereby reducing their bioactivity and the effectiveness of supplementation (Typek *et al.*, 2019; Siddiqui, 2015).

Supplementation HMB resulted in significantly lower herbal and mineral intake levels compared to mash and pellet forms (Table 3). This reduction stems from the licking based consumption method of HMB, which restricts the cattle’s ability to meet the prescribed daily doses of herbal supplements. Conversely, mineral block supplements facilitate appropriate intake levels in parts per million (ppm) when consumed through licking. This method, while effective for minerals, is insufficient for herbal supplements requiring higher consumption rates.

Despite variations in intake levels, digestibility values for dry matter, crude protein, ether extract, and other nutrients showed no significant differences across treatments (Table 4). This aligns with previous studies indicating that feed forms like mash, pellet, and HMB do not significantly alter nutrient digestibility due to comparable nutrient availability across treatments (Rostini *et al.*, 2022; Karimizadeh *et al.*, 2017). However, ADG and feed efficiency were superior in cattle fed mash compared to those fed pellet and HMB (Tables 2 and 3). This may be attributed to the rapid fermentation and lack of fibrous material in pellets, which increases the risk of acidosis (Gimeno *et al.*, 2015). Mash feed, with its smaller particle size, enhances nutrient absorption and digestion, leading to improved performance. HMB’s licking-based consumption limits herb and mineral intake, reducing its effectiveness.

Blood metabolite analysis offers insights into nutrient utilization and systemic health (Table 5). Glucose levels, initially highest in pellet treatments, became similar in mash and pellet groups by day 42, indicating an adaptive

response influenced by dietary carbohydrate content. Elevated protein levels in mash-fed cattle reflect increased bioavailability of curcumin and selenium, as curcumin closely interacts with serum proteins (Teouw and Ali, 2017). The finer particle size of mash feed allows better ruminal fermentation and enzymatic digestion, enhancing amino acid absorption and utilization for protein synthesis. This may be facilitated by its larger surface area and less dense texture, making it more easily consumed (Ebrahimi, 2020) and digested by cattle (Zaefarian *et al.*, 2016), which also results in a shorter retention time in the digestive tract. Triglyceride levels also varied, with mash showing the highest concentration on day 0, emphasizing its role in nutrient utilization.

Liver health markers, including ALT and AST levels, were significantly reduced in mash and pellet treatments, suggesting hepatoprotective effects (Table 6). These reductions can be attributed to the anti-inflammatory and antioxidant properties of curcumin and gingerol, as well as the hepatoprotective roles of zinc and selenium (Mahfudh *et al.*, 2023; Pour *et al.*, 2021). The higher ALT values observed in HMB treatments indicate stress due to insufficient herbal and mineral intake, underscoring the importance of adequate supplementation.

Haematological results (Table 7) revealed no significant differences in red blood cell counts or mean corpuscular haemoglobin values. However, higher hemoglobin (Hb), haematocrit, and mean corpuscular volume levels were observed in HMB treated cattle. This may be attributed to increased protein intake and zinc's role as a cofactor in red blood cell stabilization (Gryzbowska *et al.*, 2021). Selenium's contribution to haemoglobin stability through glutathione peroxidase further supports erythropoiesis (Kaushal *et al.*, 2011). Overall, most blood metabolites, liver enzymes, and haematological parameters remained within the normal physiological ranges for cattle. A few parameters slightly exceeded the upper reference limits, but these deviations were minor and are not considered indicative of a metabolic shift or underlying health disorder.

Adaptive immunity, as indicated by IgG titers, improved significantly in mash fed cattle by day 42. This may be attributed to the higher stability and bioactivity of curcumin and gingerol in the mash form, as they were not exposed to the thermal processing involved in pelleting (Sanidad *et al.*, 2016). Consequently, these compounds could exert their immunomodulatory effects more effectively, potentially enhancing nutrient utilization and immune responsiveness.

Furthermore, the well documented roles of curcumin and gingerol in promoting lymphocyte proliferation and

antibody production (Ko *et al.*, 2023), combined with the immune-enhancing properties of zinc and selenium, likely contributed to the superior immune responses observed in mash fed cattle. While pelleted diets provided comparable benefits, the herbal mineral block (HMB) form may have been less effective in delivering bioactive herbs and minerals, thereby limiting its immunological impact.

This study underscores the critical role of form of feed in delivering herbal-mineral supplements effectively. Mash feed demonstrated superior performance in enhancing nutrient utilization, liver health, and adaptive immunity, making it the most effective form for FMD vaccinated cattle. In contrast, HMB supplementation, while beneficial for specific minerals, was inadequate for delivering sufficient herbs, necessitating tailored approaches for optimal supplementation strategies. Future research should explore the long-term impacts of these feed forms on herd health and economic viability across diverse production systems. Further investigation into the optimal balance of herbal and mineral supplementation for various feed forms will refine strategies for improving livestock productivity.

CONCLUSION

In conclusion, mash feed is the most effective form for delivering herbal-mineral supplementation in FMD vaccinated cattle, improving average daily gain, liver health, and anti-FMD antibody titer. Future research should explore the long term impacts of these supplements on herd health and economic viability in diverse production systems.

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NOVELTY STATEMENT

To our knowledge, this is the first study to directly compare the immunomodulatory effects of herbal mineral supplementation administered in different feed forms (mash, pellet, and herbal mineral blocks) on the immune response to FMD vaccination in beef cattle. Previous research has focused mainly on growth performance or single supplementation forms, but this study integrates multiple feed delivery systems and assesses both immune parameters and metabolic profiles, offering novel insights with practical implications for ruminant nutrition and disease control.

AH: Wrote the first draft of the manuscript, conceptualized and designed the experiment; performed the study.

HR: Performed the study and data analysis.

AS and DMF: Conceptualized and designed the study, curated and validated data, reviewed and edited the manuscript.

All authors have read, reviewed and approved the final manuscript.

ETHICAL APPROVAL

The study was approved by the School of Veterinary Science and Biomedicine, IPB University (Certificate No. 143/KEH/SKE/XII/2023).

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

This manuscript was prepared with the assistance of generative AI tools, which were used exclusively to improve language clarity, grammar, and readability. All content, interpretations, and conclusions presented in this article are the sole responsibility of the authors. The authors have carefully reviewed, validated, and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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