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Dynamics of rumen microbial protein synthesis and purine derivative excretion in HF-crossbred and Deoni cows subjected to Prepartum energy fortification

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Abstract

Urinary excretion of purine derivatives is a validated, non-invasive index of rumen microbial protein supply to the host ruminant. This study used the purine derivative technique to characterise breed- and energy-source-dependent patterns of microbial protein synthesis in sixteen Holstein-Friesian crossbred and sixteen Deoni cows in a two by two by four factorial design (breed; functional nutrient supplement, present or absent; prepartum energy source: control, glycerol, jaggery, maize) at the Southern Regional Station of the National Dairy Research Institute, Bengaluru, with measurements repeated before and after calving. Urinary allantoin excretion was significantly higher in crossbred than Deoni cows before calving (4.97 versus 4.37 millimoles per litre) and after calving (4.79 versus 4.17 millimoles per litre); total purine derivative excretion, purine derivative index and duodenal microbial protein flow were likewise higher in crossbred cows in both periods ($p < 0.001$), averaging thirty-five percent higher before calving. However, when microbial protein output was expressed relative to digestible organic matter intake, the breed ranking reversed after calving; efficiency fell from eighty to forty-five grams per kilogram in crossbred cows, a forty-three percent decline, but only from seventy-three to sixty grams per kilogram in Deoni cows, an eighteen percent decline, leaving Deoni cows numerically more efficient producers per unit of fermented substrate after calving despite lower absolute output. Effects of energy source within breed were modest and largely non-significant, though efficiency rose by around seven percent in both breeds after calving with prepartum functional nutrient supplementation. Overall, the larger rumen and higher intake of crossbred cows drive greater total output, but this advantage carries a disproportionate efficiency cost during the postpartum fermentative and physiological adjustment, whereas the smaller-framed Deoni genotype maintains comparatively stable synthesis efficiency across the transition. This breed-specific pattern has direct implications for protein-sparing prepartum energy fortification strategies in tropical mixed-breed dairy herds.

Keywords: Purine derivatives, Allantoin, microbial protein synthesis, duodenal flow, rumen efficiency, transition cow

1. Introduction

Rumen microbial protein (MBP) typically supplies 50-80% of the metabolisable protein reaching the small intestine of the dairy cow, making the efficiency of its synthesis a first-order determinant of protein economy, particularly during the transition period when protein demand for colostrogenesis, fetal growth and the onset of lactation rises sharply against a background of falling or slowly recovering dry matter intake (Bach *et al.*, 2005; Hackmann and Firkins, 2015) [1, 11]. Because direct measurement of duodenal microbial flow requires cannulated animals, urinary excretion of purine derivatives (PD) — allantoin and uric acid, the end products of microbial nucleic acid degraded and absorbed in the small intestine — has become the standard non-invasive proxy for MBP supply in both research and applied settings (Chen and Gomes, 1992; Chen *et al.*, 1990) [3, 4]. Recent reviews continue to highlight that, notwithstanding its wide adoption, the PD technique carries substantial experimental error and that newer molecular approaches to characterising the rumen microbiome may eventually improve on it, though the urinary PD method remains the most practical field-applicable tool available for breed- and treatment-comparative work of the kind reported here (Lima *et al.*, 2023) [14].

Breed differences in MBP synthesis are well documented in the comparative *Bos indicus*-*Bos taurus* literature. Indicine and crossbred cattle have repeatedly been shown to differ from taurine breeds in urinary purine derivative excretion per unit of intake, in nitrogen and urea recycling, and in the endogenous purine contribution to excreted purine derivatives, reflecting differences in rumen volume, intake capacity, digesta kinetics and purine metabolism between genotypes (Zhou *et al.*, 2017; Prates *et al.*, 2012; Gandra *et al.*, 2011) [20, 17, 9]. A recent systematic review of the purine-derivative literature likewise reported systematically different purine-derivative excretion per unit of dry matter intake between *Bos indicus* and *Bos taurus* groups (Lima *et al.*, 2023) [14]. Because rumen size scales with body size and MBP output scales, in turn, with the volume of fermentable substrate processed, larger-framed crossbred cattle would be expected to produce more MBP in absolute terms than smaller indigenous cattle on a similar diet; genotype differences in digestive-tract function of this kind have been reviewed in detail elsewhere (Hegarty, 2004) [12]. Whether the larger absolute MBP output of larger-framed cattle translates into a proportionate advantage in the efficiency of MBP synthesis per unit of substrate fermented — the metric of greatest nutritional and economic relevance — is a distinct question that has received little attention in a periparturient, breed-comparative framework.

This paper addresses that question using a controlled prepartum energy-fortification trial in HF-crossbred (HFX) and Deoni cows in which urinary PD excretion, PD index, duodenal MBP flow and MBP production efficiency (g/kg digestible organic matter intake, DOMI, and g/Mcal metabolisable energy, ME) were measured in both the prepartum close-up period and again postpartum. The specific objectives were: (i) to quantify breed differences in PD excretion and MBP synthesis in each period; (ii) to characterise how prepartum energy source (control, glycerol, jaggery, maize) and functional nutrient supplementation (FNS) modulated these indices within each breed; and (iii) to compare the prepartum-to-postpartum trajectory of MBP synthesis and its efficiency between breeds, testing whether the larger absolute MBP output of HFX cows is matched by a proportionate efficiency advantage across the transition.

2. Materials and Methods

2.1 Animals, location and design

The trial was conducted at the Livestock Research Centre, ICAR-NDRI Southern Regional Station, Bengaluru. Thirty-two prepartum cows (16 HFX, 16 Deoni; 3rd-5th parity) were allocated in a 2 (breed) × 2 (FNS: with or without) × 4 (energy source: control [CG], glycerol [T1], jaggery [T2], maize [T3]) factorial design (n = 2 cows/cell) and followed from 35±5 d prepartum to 30 d postpartum. Energy sources were isocalorically substituted to supply + 10% dietary gross energy over the ICAR (2013)-balanced basal diet of mixed green fodder, ragi straw and concentrate (roughage:concentrate approximately 70:30 prepartum).

2.2 Purine derivative sampling and analysis

Spot urine samples were collected by free-catch or gentle vulval stimulation at the close-up prepartum timepoint and again during the postpartum period, and analysed for allantoin, uric acid and creatinine by standard colorimetric/HPLC-based methods following the principles

of Chen and Gomes (1992) [3]. Total PD was calculated as the sum of allantoin and uric acid; PD index was derived by relating PD concentration to estimated urine volume using the creatinine: body-weight relationship, an approach whose accuracy relative to total urine collection has been examined directly in grazing cattle (Moorby *et al.*, 2006; Da Silva Júnior *et al.*, 2021) [16, 6]; and daily PD excretion (mmol/d) and duodenal MBP flow (g/d) were computed from PD excretion using standard purine-derivative equations for cattle (Chen and Gomes, 1992; Belenguer *et al.*, 2002; González-Ronquillo *et al.*, 2003) [3, 2, 10], following the technical approach applied more recently to beef and dairy cattle by Crawford *et al.* (2020) [5] and validated for dairy diets of differing forage proportion by Moorby *et al.* (2006) [16] and for diets of differing quality by Martínez *et al.* (2010) [15]. MBP production efficiency was expressed as g MBP per kg digestible organic matter intake (DOMI) and as g MBP per Mcal metabolisable energy (ME) intake, using digestible OM and ME intake values derived from the parallel intake and digestibility measurements taken on the same animals.

2.3 Statistical analysis

Data were analysed by 2 × 2 × 4 factorial ANOVA (breed, FNS, energy source as fixed effects) separately for the prepartum and postpartum periods, with prepartum-to-postpartum change computed as the percentage difference between period means for each breed × treatment cell. Least-squares means±SEM are reported; treatment means were separated by Duncan's Multiple Range Test; significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

3. Results and Discussion

3.1 Breed differences in purine derivative excretion

Urinary allantoin excretion was significantly higher in HFX than Deoni cows in both the prepartum (4.97 vs. 4.37 mmol/L; $p < 0.01$) and postpartum (4.79 vs. 4.17 mmol/L; $p < 0.001$) periods (Table 1). Allantoin comprised a larger share of total PD in HFX cows (91% prepartum, 86% postpartum) than in Deoni cows (85% prepartum, unchanged postpartum), with the balance made up by uric acid — a pattern consistent with the 80-85%:15-20% allantoin:uric-acid partition reported for cattle generally (Chen *et al.*, 1990) [4]. Total PD concentration was higher in HFX than Deoni cows in both periods, reaching significance postpartum ($p < 0.001$) and showing a tendency prepartum ($P = 0.066$).

PD index and daily PD excretion were both significantly higher in HFX than Deoni cows in each period ($p < 0.001$), and neither showed significant interaction with energy source, FNS supplementation, or their combination — PD excretion was, in this dataset, essentially a pure breed effect, consistent with the parent thesis finding that PD excretion rate differs by breed independent of dietary energy manipulation. This is consistent with breed differences in PD excretion being driven primarily by structural factors — rumen volume, absolute digestible substrate throughput and glomerular filtration rate — rather than by the composition of prepartum energy fortification, a conclusion supported both by comparative digestion studies across Holstein, Nellore and buffalo genotypes (Gandra *et al.*, 2011) [9] and by a recent review of genotype effects on digestive-tract function in ruminants (Hegarty, 2004) [12].

3.2 Duodenal MBP flow: A rumen-size-driven breed effect

Daily duodenal MBP flow was 35% higher in HFX than Deoni cows during the prepartum period (350.1 vs. 259.1 g/d; $p < 0.001$) and remained significantly higher (43% relatively) postpartum (327.1 vs. 229.1 g/d; $p < 0.001$) (Fig. 1; Table 1). Because digestible DM or OM intake in absolute terms was consistently higher in HFX cows — despite Deoni cows showing higher intake per unit metabolic body weight, as reported in the companion comparative-intake analysis of this cohort — and because MBP synthesis scales with the absolute quantity of fermentable substrate processed rather than with substrate intake normalised to body size, the larger metabolic body size of HFX cows (26% greater than Deoni in this cohort) was the principal driver of their higher absolute MBP output. This scaling of duodenal microbial nitrogen flow with the volume of substrate processed has been demonstrated directly in calves using duodenal cannulation alongside urinary PD estimates (Funaba *et al.*, 1997) [8], and is consistent with the general principle that MBP production scales with rumen size and fermentable substrate throughput.

3.3 Energy source and functional supplement effects within breed

Across the four prepartum energy sources, PD excretion and duodenal MBP flow showed the expected breed separation within every energy group (Table 2; Fig. 3): HFX values exceeded Deoni values for allantoin, PD excretion and duodenal MBP flow at every level of energy source tested, with the gap widest under maize supplementation (500.5 vs. 344.4 mmol/d PD excretion) and narrowest under jaggery (477.3 vs. 402.4 mmol/d). MBP efficiency (g/kg DOMI) showed more overlap between breeds by energy source, with Deoni cows under jaggery (77.8 g/kg DOMI) essentially matching or exceeding HFX cows under glycerol (77.5 g/kg DOMI) — an early signal, even within the prepartum period, that Deoni MBP synthesis was not simply a scaled-down version of the HFX pattern but showed some energy-source-specific efficiency advantages of its own.

Formal ANOVA confirmed that energy source alone did not significantly affect PD excretion, PD index or duodenal MBP flow, nor did the energy $\times$ breed, energy $\times$ FNS, or three-way interactions reach significance for these traits — consistent with the parent dataset's finding that PD excretion was essentially a pure breed effect. MBP efficiency (g/kg DOMI), by contrast, did show a significant effect of energy source ($P = 0.02$ prepartum), with jaggery and maize numerically outperforming glycerol, plausibly reflecting the more rapid ruminal availability of jaggery's simple sugars and maize starch relative to the additional phosphorylation step required to route glycerol into central fermentative metabolism, a rate-limiting step also implicated in reduced ruminal digestibility responses to glycerol supplementation in cattle (Wang *et al.*, 2009) [19]. Prepartum FNS supplementation increased postpartum MBP production by approximately 7% in both breeds, though this improvement in absolute output was not matched by a statistically significant improvement in MBP efficiency, indicating that the FNS benefit operated mainly through increased substrate throughput rather than through improved conversion efficiency per unit of substrate fermented.

3.4 The prepartum-to-postpartum efficiency reversal

The most striking finding of this analysis is a reversal in relative MBP efficiency ranking between breeds across the transition (Fig. 2; Table 3). Prepartum, HFX cows were the more efficient MBP producers per unit of digestible substrate (80.0 vs. 72.7 g/kg DOMI in Deoni; $P = 0.02$) and per unit of ME intake (22.4 vs. 20.4 g/Mcal ME; $P = 0.02$). Postpartum, this ranking reversed: HFX MBP efficiency fell steeply to 45.2 g/kg DOMI — a 43% relative decline — while Deoni MBP efficiency fell only to 59.7 g/kg DOMI, an 18% relative decline, leaving Deoni cows numerically more efficient MBP producers per unit of DOMI than HFX cows in the postpartum period despite producing substantially less MBP in absolute terms (229.1 vs. 327.1 g/d). The g/Mcal ME efficiency metric did not show the same rank reversal (HFX 20.9 vs. Deoni 18.1 g/Mcal ME postpartum, a smaller relative decline of 7% in HFX vs. 11% in Deoni), indicating that the reversal is specific to the DOMI-referenced efficiency metric and reflects a disproportionate rise in digestible organic matter intake relative to microbial protein output in HFX cows after calving, rather than a uniform collapse in microbial fermentative capacity across all efficiency metrics.

This asymmetric decline is consistent with the substantially larger postpartum surge in intake documented in HFX cows in this cohort — a 74% rise in total diet DMI per unit metabolic body weight from prepartum to postpartum, against only a 12% rise in Deoni cows (reported in the companion comparative-intake analysis of this cohort) — which would be expected to push a larger, more rapidly changing volume of fermentable substrate through the rumen without a proportionate increase in microbial growth, consistent with the general principle that rapid increases in fermentable carbohydrate availability can depress microbial growth efficiency through energy-spilling and futile-cycle mechanisms in mixed rumen microbial populations (Hackmann and Firkins, 2015) [11]. This general pattern of shifting digestive and metabolic efficiency across the dry-to-lactation transition, including changing energy and nitrogen balance from the dry period into early lactation, has also been documented directly using total-collection balance trials in dairy cows (Daniel *et al.*, 2022) [7]. All postpartum MBP efficiency values in both breeds nonetheless remained well above the approximately 30 g/kg DOM optimum reported for continuous-culture rumen fermentation systems (Hackmann and Firkins, 2015) [11], indicating that neither breed's rumen microbial population was functioning inefficiently in absolute terms — the reversal is a relative, between-breed phenomenon rather than evidence of dysfunction in either group.

Both allantoin and total PD showed relatively small prepartum-to-postpartum declines (Fig. 4; Table 3) in contrast to the much larger declines in PD index, PD excretion, duodenal MBP flow and MBP efficiency, a pattern attributable in part to elevated prepartum plasma and urinary allantoin arising from fetal and whole-body nucleic acid turnover in the last weeks of gestation, a phenomenon reported to inflate apparent MBP estimates during the final eight weeks of pregnancy before falling sharply after calving. Creatinine and uric acid excretion rose more in HFX (+ 26% and + 65%, respectively) than in Deoni cows (+ 8% each) from prepartum to postpartum, consistent with the larger increase in muscle metabolic turnover and absolute digestible substrate flow accompanying the

substantially larger postpartum intake surge in HFX cows documented above.

3.5 Integrative interpretation and practical implications

Taken together, these results indicate that breed shapes microbial protein synthesis in two distinct ways that point in different directions. In absolute terms, the larger-framed HFX genotype consistently out-produced the smaller Deoni genotype in PD excretion and duodenal MBP flow in both the prepartum and postpartum periods, a rumen-size and substrate-throughput effect that is unsurprising and consistent with the wider comparative-nutrition literature. In relative, efficiency terms, however, the postpartum period exposed an asymmetry: HFX cows paid a much larger efficiency penalty (43% decline in g/kg DOMI) than Deoni cows (18% decline) for their much larger postpartum intake surge, to the point of falling behind Deoni cows in DOMI-referenced MBP efficiency after calving despite having started the prepartum period with a significant efficiency advantage.

From a practical protein-nutrition standpoint, this suggests that the rapid postpartum ration changes typically applied to high-yielding crossbred cows to meet their much larger absolute nutrient demand may be outpacing the rumen microbial population's capacity to convert that additional substrate into protein with matching efficiency, at least during the acute early-lactation adjustment window captured here. Where feasible, a more graduated postpartum transition in fermentable carbohydrate and protein supply for HFX-type cows — rather than the sharp step-up documented in this cohort — could help narrow the efficiency penalty and reduce the volume of degraded

protein and carbohydrate lost to inefficient microbial metabolism rather than converted to usable microbial protein. For indigenous Deoni cows, by contrast, the comparatively stable MBP efficiency across the transition suggests less urgency for postpartum ration graduation on this specific axis, consistent with the more modest and better-matched postpartum intake response documented in this breed.

Limitations should be noted. PD-based MBP estimates in the prepartum period are subject to a well-documented upward bias from fetal and whole-body nucleic acid turnover in late gestation, meaning that absolute prepartum MBP efficiency values (up to 80 g/kg DOMI, well above even the top of the theoretical *in vivo* range of 34-66% of maximum microbial growth efficiency reported elsewhere (Hackmann and Firkins, 2015))^[11] should be interpreted as relative rather than absolute estimates, as is standard practice in the PD-technique literature (Chen and Gomes, 1992)^[3]. Because spot urine sampling and the creatinine:body-weight assumption were used to estimate daily urine volume rather than total urine collection, some estimation error is expected; work directly comparing spot-sample and total-collection PD estimates, and comparing collection days and times, indicates that such error, while non-trivial, would be expected to affect both breeds similarly and should not materially alter the relative breed comparison that is the focus of this paper (Da Silva Júnior *et al.*, 2021; Silva Júnior *et al.*, 2018)^[6, 18]. A dedicated study incorporating total urine collection and duodenal cannulation across the transition period would be needed to confirm the magnitude of the efficiency reversal identified here with greater precision.

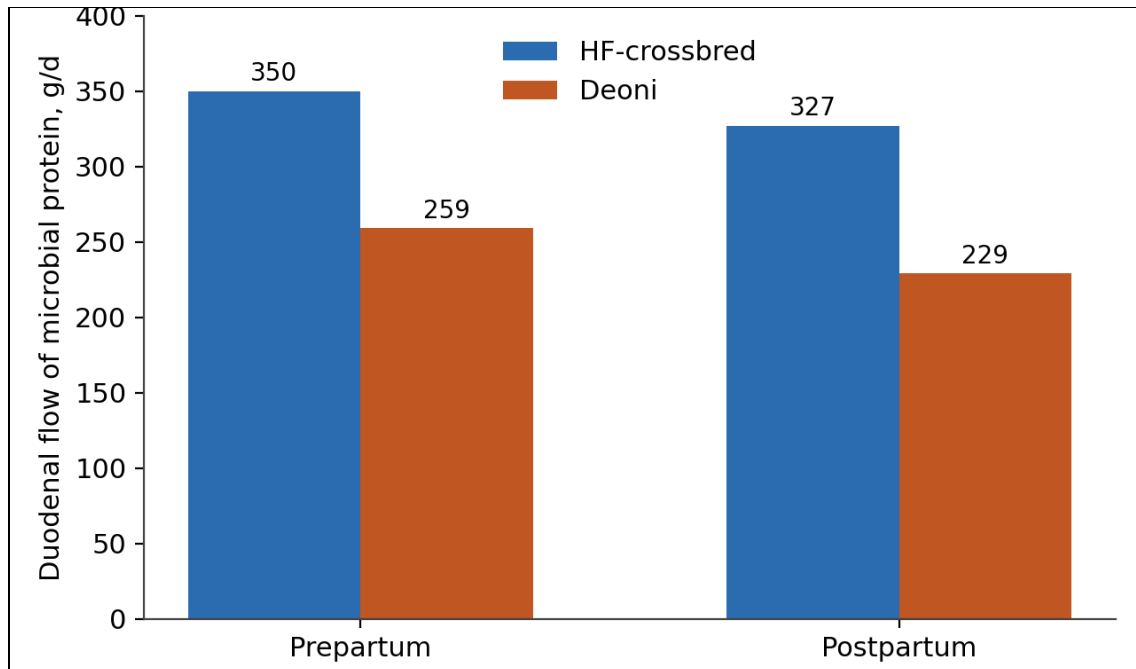


Fig 1: Duodenal microbial protein (MBP) flow (g/d) by breed and physiological period. HFX cows produced significantly more MBP in absolute terms than Deoni cows in both periods ($p < 0.001$).

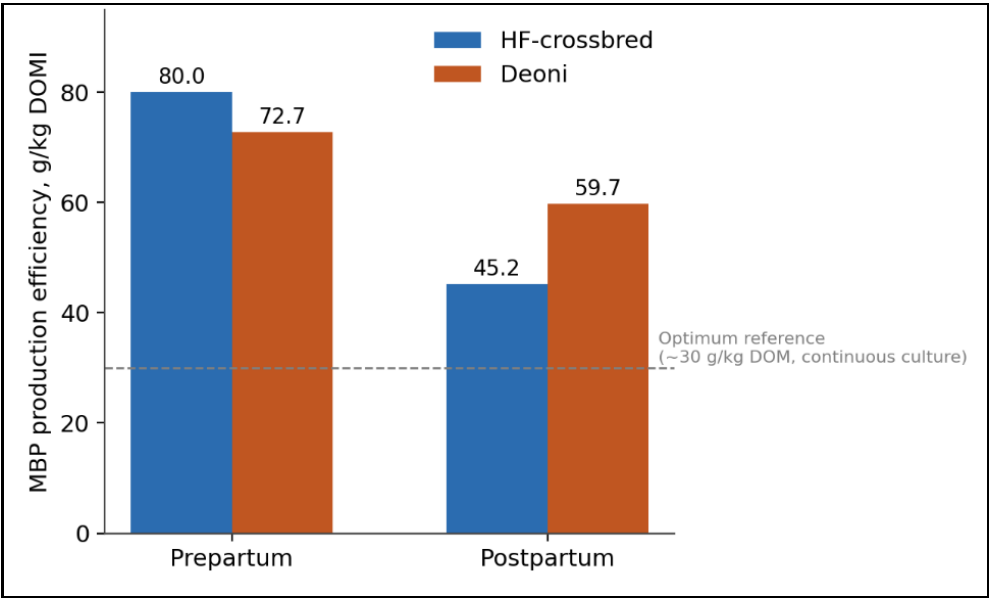


Fig 2: MBP production efficiency (g/kg DOMI) by breed and physiological period, showing the prepartum HFX advantage reversing to a Deoni advantage postpartum. Dashed line: ~30 g/kg DOM optimum reported for continuous-culture rumen fermentation.

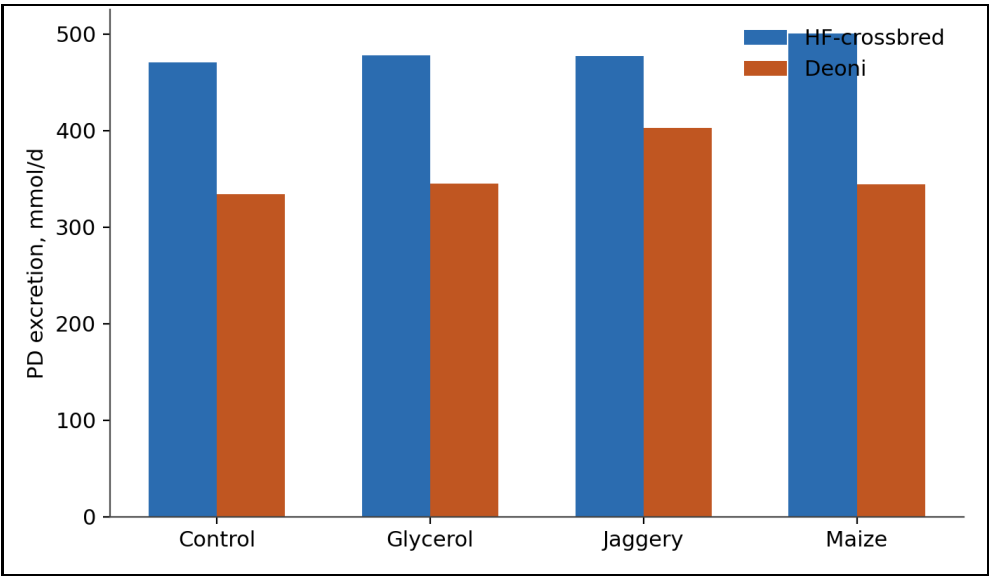


Fig 3: Prepartum urinary purine derivative (PD) excretion (mmol/d) by breed and prepartum energy source (Control, Glycerol, Jaggery, Maize).

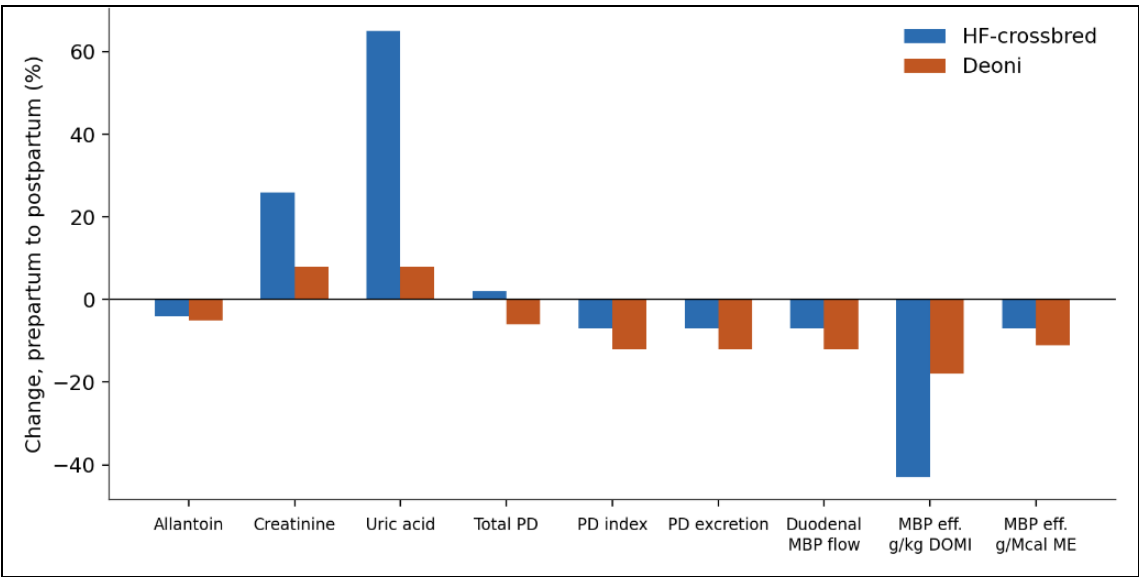


Fig 4: Breed contrast in percentage change from prepartum to postpartum across purine derivative and MBP indices.

Table 1: Breed means for purine derivative and microbial protein indices, prepartum and postpartum periods

Parameter	Period	HF-crossbred	Deoni	P-value (breed)
Allantoin, mmol/L	Prepartum	4.97	4.37	$p < 0.01$
	Postpartum	4.79	4.17	$p < 0.001$
Creatinine, mmol/L	Prepartum	2.12	1.89	NS ($P = 0.17$)
	Postpartum	2.67	2.05	$P = 0.001$
Uric acid, mmol/L	Prepartum	0.43	0.65	NS ($P = 0.19$)
	Postpartum	0.71	0.70	NS ($P = 0.74$)
Total PD, mmol/L	Prepartum	5.40	5.17	Tendency ($P = 0.066$)
	Postpartum	5.51	4.86	$p < 0.001$
PD index	Prepartum	548.8	405.3	$p < 0.001$
	Postpartum	513.1	357.9	$p < 0.001$
PD excretion, mmol/d	Prepartum	481.5	356.3	$p < 0.001$
	Postpartum	449.9	315.1	$p < 0.001$
Duodenal MBP flow, g/d	Prepartum	350.1	259.1	$p < 0.001$
	Postpartum	327.1	229.1	$p < 0.001$
MBP efficiency, g/kg DOMI	Prepartum	80.0	72.7	$P = 0.02$
	Postpartum	45.2	59.7	$p < 0.001$
MBP efficiency, g/Mcal ME	Prepartum	22.4	20.4	$P = 0.02$
	Postpartum	20.9	18.1	$p < 0.001$

Table 2: Prepartum purine derivative excretion and MBP flow by breed and energy source

Energy source	Breed	Allantoin (mmol/L)	PD excretion (mmol/d)	Duodenal MBP (g/d)	MBP eff. (g/kg DOMI)
Control	HFX	5.19	470.6	342.2	86.9
Control	Deoni	4.56	334.0	242.8	74.7
Glycerol	HFX	4.83	477.5	347.1	77.5
Glycerol	Deoni	4.25	344.6	250.5	66.9
Jaggery	HFX	4.87	477.3	347.0	76.3
Jaggery	Deoni	4.33	402.4	292.6	77.8
Maize	HFX	5.00	500.5	363.9	79.2
Maize	Deoni	4.34	344.4	250.4	71.6

Table 3: Prepartum-to-postpartum percentage change in purine derivative and MBP indices, by breed

Index	HF-crossbred,%	Deoni,%
Allantoin	-4	-5
Creatinine	+ 26	+ 8
Uric acid	+ 65	+ 8
Total PD	+ 2	-6
PD index	-7	-12
PD excretion	-7	-12
Duodenal MBP flow	-7	-12
MBP efficiency, g/kg DOMI	-43	-18
MBP efficiency, g/Mcal ME	-7	-11

4. Conclusion

HF-crossbred cows produced significantly more microbial protein than Deoni cows in absolute terms throughout the transition period, consistent with their larger rumen size and greater absolute digestible substrate intake. However, this absolute advantage was not matched by a proportionate efficiency advantage after calving: MBP production efficiency per unit of digestible organic matter intake declined by 43% in HFX cows postpartum against only 18% in Deoni cows, reversing the prepartum efficiency ranking between breeds. Energy source had limited effect on PD excretion or duodenal MBP flow within breed, but did significantly affect MBP efficiency, and prepartum functional nutrient supplementation modestly increased postpartum MBP output in both breeds. These findings identify the postpartum period as a window of disproportionate microbial-protein-synthesis efficiency cost in high-yielding crossbred cows relative to indigenous cattle, and support more graduated postpartum ration

transitions as a potential strategy to narrow this efficiency gap in tropical mixed-breed dairy systems.

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