Research Article

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Effect of Monensin Sodium Supplementation on Production Performance in Lactating Cattle

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The present study was undertaken to evaluate the effect of monensin sodium supplementation on nutrient utilization, metabolic response, blood biochemical profile, and production performance in lactating Sahiwal cattle. Eighteen Sahiwal cows of similar body weight and milk yield were randomly allocated into three dietary groups: Control (basal diet without monensin sodium: Ms0), Treatment-1 (basal diet with monensin sodium @ 150 mg/animal/day: MS_{150}), and Treatment-2 (basal diet with monensin sodium @ 300 mg/animal/day: MS_{300}). All animals were offered a uniform basal ration containing 60% concentrate and 40% wheat straw formulated to meet nutrient requirements. Daily dry matter intake and fortnightly body weight were recorded to assess changes in performance. Dry matter intake (kg/day and % BW) and metabolic body weight remained statistically similar among groups. Digestibility coefficients of Dry Matter (DM), Crude Fiber (CF), Ether Extract (EE), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were comparable across treatments with no significant difference in treatment groups; however, organic matter and crude protein digestibility were significantly enhanced in both monensin sodium supplemented groups. Haematological parameters remained within normal physiological ranges and were not impacted by supplementation of different levels of monensin sodium. Among blood metabolites, glucose and total protein showed a marked significant improvement while β-hydroxybutyrate (BHBA) levels showed a significant decrease. Plasma minerals (Ca, P, Cu, Zn, Mn) remained unaffected with supplementation of monensin sodium. Milk yield improved significantly in cows receiving 300 mg/day monensin sodium (MS_{300}), while composition parameters like milk fat, protein, lactose, and SNF remained unchanged with monensin sodium supplementation. It may be concluded that monensin sodium, at an optimal dose of 300 mg/animal/day (MS_{300}), improves nutrient digestibility, enhances metabolic efficiency and increases milk yield without adverse effects on animal health.

Keywords : Monensin, Ionophore, Sahiwal, Lactating, Milk production

Introduction

India possesses one of the largest livestock populations globally, and the livestock sector plays a vital role in improving rural livelihoods (DAHD, 2023). Although India is the world's leading milk producer, average milk yield per animal remains substantially lower than that of developed countries, mainly due to inefficient nutrient utilization (Ahvanooei et al., 2023). Inefficient rumen fermentation results in greater losses of feed energy as methane and nitrogen as ammonia, negatively affecting production efficiency and contributing to greenhouse gas emissions (Mammi et al., 2021). Consequently, nutritional strategies aimed at manipulating rumen microbial ecology have gained renewed attention in recent years as a sustainable approach to enhance productivity and reduce enteric methane emissions (Ahvanooei et al., 2023). Among feed additives, ionophore antibiotics remain one of the most effective tools for improving rumen efficiency. Ionophores such as monensin, lasalocid, laidlomycin, salinomycin, and narasin are commonly offered to ruminant animals to increase feed efficiency. Monensin is one of the most regularly utilised ionophores (Tyler et al., 1992). Monensin sodium, a polyether ionophore produced by *Streptomyces cinnamonensis*, selectively inhibits Gram-positive rumen bacteria, resulting in increased propionate production and reduced acetate, butyrate, methane, and ammonia synthesis (Trautmann et al., 2023). Monensin supplementation can reduce ruminal acetate to propionate ratio, enteric methane, and ammonia generation, while also improving feed efficiency and daily weight gain (Hemphill et al., 2018). Monensin reduces ammonia and lactate synthesis while increasing propionate production efficiency of energy metabolism, hence enhancing nitrogen metabolism by specifically inhibiting Gram-positive bacteria (Chalupa, 1980; McGuffey et al., 2001). Recent studies have demonstrated that monensin supplementation can improve milk yield, feed efficiency, and metabolic health in lactating cattle, with modest or no adverse effects on dry matter intake (DMI) when appropriately dosed (Gonzalez-Chappe et al., 2025).

Materials and Methods

The present study was conducted to evaluate the effect of monensin sodium supplementation on production performance, nutrient utilization, and blood biochemical parameters in lactating indigenous cattle.

Ethical Approval

The experiment was carried out at the Livestock Farm Complex (LFC), DUVASU, Mathura. Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC), DUVASU, Mathura (Approval No. IAEC/25/59), constituted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

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Animals Detail

Eighteen lactating Sahiwal cows were selected from the institute herd and housed individually in a well-ventilated shed with separate arrangements for feeding and watering, ensuring no access to other animals' diets. The sheds were cleaned daily to maintain hygienic conditions. All animals were dewormed prior to the commencement of the experiment using fenbendazole and ivermectin, and were allowed weekly exercise. The experimental animals were randomly allotted into three groups of six animals each.

Feeding and Management

The control group (MS_0) was fed a basal diet without monensin sodium supplementation, whereas the treatment groups (MS_{150} & MS_{300}) received monensin sodium at 150 mg/animal/day/and 300 mg/animal/day, respectively. All animals were fed a basal ration consisting of wheat straw and a compounded concentrate mixture formulated to meet nutrient requirements as per NRC (2001). The concentrate mixture was prepared using maize grain, barley grain, wheat bran, gram chunni, mustard oil cake, cottonseed cake, and mineral mixture in fixed proportions. Monensin sodium was premixed thoroughly with the concentrate mixture and offered individually to animals according to their respective treatment groups. Ingredients and chemical composition (% DM basis) of total mixed ration (TMR) fed during experimental period are presented in Table 1 & 2. DMI was recorded daily by measuring the quantity of feed offered and the residue left. Body weight of experimental animals was recorded at the beginning of the experiment and subsequently at fortnightly intervals before feeding and watering.

Digestion Trial

Digestion trial of seven days was conducted at the end of the feeding experiment to assess nutrient utilization. During the digestion trial, weighed quantities of feed were offered, and total faeces voided over 24 hours were collected daily. Representative samples of feed, refusals and faeces were processed for chemical analysis, and apparent digestibility coefficients were calculated based on nutrient intake and faecal output.

Chemical Analysis

Feeds, residue and faecal samples were dried, ground, and analysed for dry matter, organic matter, crude protein, ether extract, crude fibre, and total ash following standard AOAC (2005) procedures. Fibre fractions including neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and hemicellulose were estimated as per the method described by Van Soest et al. (1991). Lactating cows were milked twice daily, and milk yield was recorded daily using a digital weighing balance. Milk samples were collected at fortnightly intervals during morning milking and analysed for fat, protein, lactose and solids-not-fat using a Lactoscan milk analyser MCC WS made by MILKOTRONIC LTD, Bulgaria.

Table 1. Ingredients composition (% DM basis) of total mixed ration (TMR) fed during experimental period

Ingredients (%)	Supplemental Monensin Sodium (mg/animal/day)		
	Ms_0	Ms_{150}	MS_{300}
Maize grain	21	21	21
Barley grain	16	16	16
Wheat bran	15	15	15
Gram chunni	16	16	16
Mustard oil cake	10	10	10
Mineral mixture	02	02	02
Cotton Seed cake	20.00	20.00	20.00
Wheat straw	40.00	40.00	40.00

Table 2. Chemical composition (% DM basis) of nutrients of total mixed ration (TMR) fed during experimental period

Ingredients (%)	Supplemental Monensin Sodium (mg/animal/day)		
	Ms_0	Ms_{150}	MS_{300}
Nutrients	Chemical composition (%)		
DM	91.80	91.80	91.80
OM	91.05	91.05	91.05
EE	3.15	3.15	3.15
CP	13.80	13.80	13.80
ASH	8.97	8.97	8.97
CF	23.31	23.31	23.31
NFE	50.17	50.17	50.17
NDF	61.99	61.99	61.99
ADF	29.88	29.88	29.88
ADL	1.70	1.70	1.70
Cellulose	28.19	28.19	28.19
Ca (%)	1.16	1.16	1.16
P (%)	0.56	0.56	0.56

DM=Dry Matter; OM=Organic Matter; EE=Ether Extract; CP=Crude Protein; CF=Crude Fiber; NFE=Nitrogen Free Extract; NDF=Neutral Detergent Fiber; ADF=Acide Detergent Fiber; ADL=Acid Detergent Lignin;

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Blood samples were collected at 0, 30, and 60 days before feeding and watering. Whole blood was used for haematological analysis, while plasma was separated and stored at −20 °C for biochemical estimations. Haematological attributes (Hb, PCV, RBCs, WBCs, MCV, MCHC and Platelets) in blood were analyzed by Celltac-á (MEK-6500K) auto haemoanalyser made by Nihon Kohden, Surat, India. The plasma concentration of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), bilirubin, creatinine, total protein, albumin and Plasma Urea Nitrogen (PUN), triacylglycerides, cholesterol and glucose were determined by using automated biochemical analyzer using commercial kits (Span Diagnostic Ltd. Surat, India). Plasma â-hydroxybutyric acid (BHBA) and insulin were determined in plasma by using bovine-specific ELISA kits (Bioassay Technologies, China). Plasma mineral concentrations were analysed following microwave digestion (Microwave digestion system: Multiwave 5000, Anton Paar, Virginia, USA) using inductively coupled plasma optical emission spectroscopy (ICP).

Statistical Analysis

The data for measured variables were subjected to analysis of variance (ANOVA) using the mixed model repeated measure procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA). Individual animal was used as the experimental unit for all data. The statistical difference between the means was determined by using ''Tukey's honest significant difference (HSD) test.''

Results

Effect on intake and growth performance

Effect of monensin sodium supplementation on DMI and growth performance are depicted in Table 3. Daily DMI (kg/d) in MS_0, MS_{150} and MS_{300} groups during the experimental feeding was recorded and statistical analysis of data revealed that variation among the groups for DMI was not significantly different (P=0.635) between the groups and the mean daily DMI in the MS_0, MS_{150} and MS_{300} groups was 9.83, 9.39 and 9.45 kg/day. BW gain of Lactating cattle in 3 different groups measured at fortnightly intervals and found no effects of treatment on the fortnightly BW change. There was also no significant (P=0.971) effect of treatment on the fortnightly BW gain during the experimental period.

Table 3. Effect of monensin sodium supplementation on growth performance, intake and nutrients digestibility

Attributes	Supplemental Monensin Sodium (mg/animal/day)			SEM	Significance
	Ms_0	Ms_{150}	MS_{300}		
BW change (kg)	350.98	353.88	353.36	5.13	0.971
BW change (kg $^{W0.75}$)	80.99	81.46	81.29	0.88	0.977
Daily DMI (kg/day)	9.83	9.39	9.45	0.19	0.635
Nutrient Digestibility (%)					
DM	54.14	55.06	56.19	0.60	0.388
OM	62.55^{a}	64.23^{ab}	65.89^{b}	0.54	0.044
CP	64.31^{a}	69.19^{b}	68.41^{b}	0.65	0.004
CF	52.60	50.31	53.63	0.83	0.407
EE	76.20	76.38	77.76	0.33	0.119
NFE	68.10	70.34	69.10	0.46	0.136
NDF	54.44	55.42	57.69	0.61	0.076
ADF	41.66	39.19	44.73	1.04	0.096

DM=Dry Matter; OM=Organic Matter; CP=Crude Protein; CF=Crude Fiber; EE=Ether Extract; NFE=Nitrogen Free Extract; NDF=Neutral Detergent Fiber; ADF=Acide Detergent Fiber. Means within a row with different superscript letters differ significantly (P<0.05).

Effect on nutrient utilization

Effect of monensin sodium supplementation on nutrients digestibility are depicted in Table 3. The digestibility coefficients of DM, EE, NFE were unaffected by different levels of monensin sodium supplementation. However organic matter and crude protein digestibility increased significantly in monensin-fed groups, particularly in 300 mg/day group.

Effect on milk production and milk composition

The effect of monensin sodium supplementation on milk yield (kg/d), milk fat (%), milk protein (%), milk lactose (%) and milk SNF (%) of experimental animals is presented in Table 4. During the experimental period, milk production showed a significant (P<0.05). improvement in the MS_{300} group compared to MS_0 and MS_{150}. The mean daily milk yield was 6.15, 6.18, and 6.57 kg/d for MS_0, MS_{150} and MS_{300} groups, respectively. The mean fat percentage across the study period was 4.24, 4.17, and 4.09% in MS_0, MS_{150} and MS_{300} groups, respectively. statistical analysis revealed no significant difference (P>0.05) among the groups. Across the experimental period, milk protein percentage remained relatively stable, with mean values of 3.29, 3.25, and 3.26% in respective groups. No significant variation (P>0.05) was detected among treatments, suggesting that inclusion of monensin sodium in the diet did not alter milk protein content The mean lactose percentage was 4.93, 4.92, and 4.92% for MS_0, MS_{150} and MS_{300} groups, respectively. Statistical analysis revealed no significant difference (P>0.05) among the groups. The mean SNF percentage for the overall experimental period was 8.99, 8.94, and 8.93% in the MS_0, MS_{150} and MS_{300} groups, respectively, showing a similar non-significant trend among all treatments.

Effect on blood biomarkers

Effect of monensin sodium supplementation on haematology profile is showed in Table 5. Hematological parameters RBC, WBC, Hb, PCV, MCV, MCHC and platelets showed no significant variation among groups in the present study.

Effect of monensin sodium supplementation on plasma mineral concentration is showed in Table 5. Based on the overall mean values, monensin sodium supplementation did not exert any significant influence on the plasma mineral profile of lactating Sahiwal cattle throughout the experimental period. Overall, the consistency of both macro and micro mineral concentrations within normal physiological ranges suggests that supplementation of

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monensin sodium at either 150 or 300 mg/animal/day did not disturb mineral metabolism, absorption, or homeostasis in lactating cattle under the conditions of the present study. Similarly, plasma Zn, Cu, and Mn concentrations showed no significant differences among groups in the present study, indicating that monensin did not alter trace-mineral status under the applied dietary conditions.

Effect of monensin sodium supplementation on blood biochemicals are presented in table 6. The feeding of a diet supplemented with monensin sodium observed significant treatment effect for plasma glucose concentration (P<0.05). The overall mean values across the 60-day study averaged 63.19, 68.03 and 72.07 mg/dL in MS_0, MS_{150} and MS_{300} groups, respectively. The feeding of a diet supplemented with monensin sodium observed significant treatment effect for plasma total protein concentration (P<0.05). The overall mean values across the 60-day study averaged 6.55, 6.69 and 6.81 g/dL in MS_0, MS_{150} and MS_{300} groups, respectively. In present study, plasma cholesterol, triglycerides, albumin and globulin concentration remained statistically similar among treatments. In the present study, â-hydroxybutyrate (BHB) concentration was significantly lower in monensin-supplemented groups, especially at 300 mg/day, indicating improved energy balance and reduced ketone body accumulation. The overall mean BHBA (â-hydroxybutyric acid) concentration was 0.77 and 0.82 in MS_0 and MS_{150} groups, respectively, while a lower value of 0.52 was recorded in MS_{300} group indicating a noticeable reduction in BHBA levels in the monensin sodium supplemented groups. Liver enzymes (ALT, AST, ALP and Bilirubin) and renal indicators (creatinine, PUN) remained within normal physiological range in all treatment groups, exhibiting no negative response to monensin supplementation.

Discussion

Effect on Intake and Growth Performance

Statistical analysis of data revealed that variation among the groups for daily DMI was not significantly different (P=0.635) among the different groups (MS_0, MS_{150} and MS_{300}). A comparable non-significant (P=0.247) intake trend was reported by Duffield et al. (2000) in Holstein cows supplemented with monensin at 200 mg/day, wherein intake remained stable despite improved production efficiency. Similar observations were presented by Ahvanooei et al. (2023) in a study involving 48 early-lactation cows offered monensin through TMR, where DMI remained statistically unaffected. Similar to the current study Mullins et al. (2012) observed that DMI in pre and postpartum period was not affected. A variety of factors, including the number of animals in the trial, the lactation stage, and the duration of data collection, could account for the varying response to monensin supplementation in the DMI. Monensin improves rumen fermentation efficiency without causing measurable changes in intake, supporting the findings of the current study. The increased propionate production driven by monensin influences satiety and also DMI. Contrary to this, Navarro et al. (2019) conducted a 72-day study on 24 lactating Jersey cows supplemented with gradual monensin levels and recorded a significant decline in feed intake during peak lactation. BW gain of lactating cattle in 3 different groups measured at fortnightly intervals and found no effects of treatment on the fortnightly BW

Table 4: Effect of monensin sodium supplementation on milk yield and milk composition

Attributes	Supplemental Monensin Sodium (mg/animal/day)			SEM	Significance		
	Ms_0	Ms_{150}	MS_{300}		Treatment (T)	Period (P)	T×P
Milk yield (Kg/d)	6.15[a]	6.18[a]	6.57[b]	0.05	0.041	0.367	0.624
Milk fat (%)	4.24	4.17	4.09	0.05	0.263	0.984	0.961
Milk protein (%)	3.29	3.25	3.26	0.01	0.322	0.977	0.893
Milk lactose (%)	4.93	4.92	4.92	0.02	0.600	0.907	0.899
Milk SNF (%)	8.99	8.94	8.93	0.26	0.383	0.917	0.846

SNF=Solid Not Fat. Means within a row with different superscript letters differ significantly (P<0.05).

Table 5. Effect of monensin sodium supplementation on haematology profile and plasma mineral concentration

Attributes	Supplemental Monensin Sodium (mg/animal/day)			SEM	Significance		
	Ms_0	Ms_{150}	MS_{300}		Treatment (T)	Period (P)	T×P
Haematology Profile							
RBCs (10^6/µL)	5.51	5.95	5.37	0.10	0.601	0.854	0.890
WBCs (10^3/µL)	10.31	9.07	9.15	0.29	0.120	0.935	0.942
Hb (g/dL)	8.91	9.08	8.59	0.13	0.076	0.870	0.937
HCT (%)	25.73	26.81	24.04	0.54	0.301	0.888	0.910
MCV (fL)	46.72	45.53	45.54	0.66	0.496	0.996	0.997
MCHC (g/dL)	33.38	33.38	33.17	0.07	0.312	0.827	0.289
Platelets (10^3/µl)	211.61	243.61	249.55	8.97	0.082	0.828	0.386
Plasma mineral concentration							
Calcium (mg/dL)	9.73	9.45	9.62	0.17	0.802	0.853	0.561
Phosphorous (mg/dL)	5.59	5.51	5.60	0.03	0.207	0.851	0.538
Copper (mg/L)	1.16	1.12	1.09	0.02	0.805	0.079	0.256
Zinc (mg/L)	1.19	1.15	1.17	0.02	0.961	0.237	0.145
Manganese (µg/L)	2.56	2.17	2.50	0.03	0.943	0.205	0.992

HCT=Haematocrt Value; MCV=Mean Corpuscular Volume; MCHC=Mean Corpuscular Haemoglobin Concentration. Means within a row with different superscript letters differ significantly (P< 0.05).

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change. There was also no significant (P=0.971) effect of treatment on the fortnightly BW gain during the experimental period. There was no significant difference in BW observed on supplementation of different levels of monensin sodium in diet of cattle. Comparable outcomes were obtained by Ipharraguerre and Clark (2003) who supplemented (335 mg/cow/day) monensin in 60 lactating Holstein cows for 120 days with no significant change in BW. Similarly, Penner et al. (2014) evaluated monensin inclusion in 36 multiparous cows and reported no appreciable variation in body mass despite higher energy extraction. In contrast, Makwana et al. (2022) observed slight BW gain when 24 Gir cows were supplemented with monensin for 75 days. This improvement is attributed to the reduction in methane energy losses and the increased capture of dietary energy as metabolizable energy. Based on the potential of ionophores to increase the supply of glucogenic precursors such as propionate, the administration of monensin to dairy cows may increase the hepatic synthesis of glucose and, therefore, improve the energy balance.

Effect on nutrient utilization

Effect of monensin sodium supplementation on nutrients digestibility are depicted in Table 3. The digestibility coefficients of DM, EE, NFE were unaffected by different levels of monensin sodium supplementation. However organic matter and crude protein digestibility increased significantly in monensin-fed groups, particularly in 300 mg/day group. This agrees with McGuffey et al. (2001) who reported increased OM and CP utilization in dairy cattle receiving monensin-based diets. Additionally, Souri et al. (2022) evaluated monensin in 12 multiparous cows over 60 days and documented a similar rise in digestibility coefficients, supporting the findings in this study. The improvement of digestibility of organic matter in T2 collaborates with finding of Sengupta et al. (2017). While the improvement in crude protein digestibility was similar to findings of Ipharraguerre and Clark. (2003). This improvement can be attributed by effect of monensin sodium supplementation on gram positive bacteria which are sensitive to monensin and indirectly reduce rumen ammonia concentration. However, contrasting responses have been observed in some species. Saeed et al. (2020) supplemented monensin to 30 lambs over 90 days and reported numerical but non-significant improvements in OM and CP digestion. In another comparable trial, Tian et al. (2021) supplemented monensin to 36 buffaloes and reported significant improvements in CP degradation but no change in OM utilization.

Fiber fractions (NDF, ADF and CF) did not significantly differ among groups, matching results of Kononoff et al. (2006) in Holstein cows. Xia et al. (2020) in a 60-day study on lactating buffaloes also reported no improvement in cell-wall digestion with monensin. Together, these findings demonstrate consistency in fiber retention patterns and show that monensin primarily enhances fermentable nutrient digestion rather than structural carbohydrates. However, Ahvanooei et al. (2023) observed adding monensin enhanced fibre digestion without having any adverse effect on ADF.

Effect on milk production and milk composition

During the experimental period, milk production showed a significant (P<0.05). improvement in the MS_{300} group compared to MS_0 and MS_{150} (Table 4). Similar results were found by Ueda et al. (2003), reported increased yield in monensin-fed Holstein cows, aligning precisely with our results. Schultz et al. (2006) also recorded dose-responsive improvement in milk, closely reflecting the T2 response. Yadav et al. (2023) noted that monensin supplementation in early-lactating buffaloes (400 mg/day) reduced DMI but improved milk production and altered milk fat composition, milk yield increased by approximately 0.59 kg/day compared to control group. Phipps et al. (2000) examined the effects of monensin on milk output and feed intake in two studies with Holstein-Friesian cows given completely varied menus. In experiment 1. When compared to the control cows, the milk yield response of 2.8 and 2.5 kg/d that occurred when cows received 150 and 300 mg/d of monensin was significant (P<0.05). 1.5 kg/d increase in milk yield from the use of the highest dose rate (300 mg/d), there was no discernible difference between the control and the two lower dose rates. While experiment 2 observed monensin raised milk yield in T1 and T2 by 0.8 and 1.1 kg/d, respectively as compared to control. Similarly, Fernandes et al. (2020) supplemented monensin to 30 Murrah buffaloes for 56 days and recorded a 9-12% yield increment, which resembles the rise recorded in the present study in Sahiwal cows. Meanwhile, Konwar et al. (2019) observed no significant increase when monensin was fed to Gir × HF crossbreds, indicating that genetic background may govern response magnitude.

Across the experimental period, milk fat, protein, lactose and SNF percentage remained relatively stable, among the groups. No significant variation (P>0.05) was detected among treatments, suggesting that inclusion of monensin sodium in the diet did not alter the concentration of these components of milk. Statistical analysis revealed no significant difference (P>0.05) among the groups. It was similar to findings of Gupta et al. (2018) who evaluated effect of dietary monensin supplementation of 12 lactating buffaloes which was allocated to two groups one without monensin supplementation and other with monensin 24 mg/kg of DMI for 60 days, in this study it was evaluated that the average milk fat, milk protein, milk lactose and milk solid not fat were similar in both groups. Similarly, Ipharraguerre and Clark (2003) documented identical results in monensin-supplemented Holstein cows. Grainger et al. (2010) reported similar SNF and protein stability in monensin-based feeding trials. In a contrasting observation, Sadri et al. (2023) reported a decrease in milk fat in high-dose monensin cows, although total milk output improved.

Effect on blood biomarkers

Effect of monensin sodium supplementation on haematology profile is showed in Table 5. Hematological parameters RBC, WBC, Hb, PCV, MCV, MCHC and platelets showed no significant variation among groups in the present study. Similar outcomes were reported by Muthy et al. (2018) when monensin was supplemented in growing cattle. Likewise, Hidalgo et al. (2020) conducted a 10-week trial on 27 multiparous cows and reported no hematological changes under monensin inclusion. Conversely, Anwar et al. (2022) supplemented monensin sodium to 18 crossbred calves and observed elevated lymphocyte counts, suggesting additive-specific immune modulation. No such deviation occurred in the present study, indicating monensin's hematological stability.

Effect of monensin sodium supplementation on plasma mineral concentration is showed in Table 5. Based on the overall mean values, monensin sodium supplementation did not exert any significant influence on the plasma mineral profile of lactating Sahiwal cattle throughout the experimental period. Overall, the consistency of both macro and micro mineral concentrations within normal physiological ranges suggests that supplementation of monensin sodium at either 150 or 300 mg/animal/day did not disturb mineral metabolism, absorption, or homeostasis in lactating cattle under the conditions of the present study. Calcium and phosphorus concentrations remained statistically unchanged. Nagaraja et al. (1997) reported similar Ca-P stability in ionophore-fed cattle, reinforcing our findings. Smith et al. (2021) in dairy

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heifers also observed no significant fluctuations in mineral homeostasis under monensin, confirming that monensin does not alter mineral absorption pathways. Similarly, plasma Zn, Cu, and Mn concentrations showed no significant differences among groups in the present study, indicating that monensin did not alter trace-mineral status under the applied dietary conditions. Similar outcomes were noted by Beckett et al. (1998), who reported that monensin inclusion did not produce measurable changes in plasma Cu or Zn in dairy cows.

Effect of monensin sodium supplementation on blood biochemicals are presented in table 6. The feeding of a diet supplemented with monensin sodium observed significant treatment effect for plasma glucose concentration (P<0.05). These findings align with Bauer et al. (1995) who reported enhanced metabolic glucose in cows receiving ionophore supplementation. Mammi et al. (2021) found that monensin supplementation with 300–400 mg/day of monensin boosts glucose availability by boosting propionate synthesis in the rumen, a key gluconeogenic precursor, monensin supplementation in the trial elevated blood glucose levels by 10-15% during early lactation phase. The increased plasma glucose can be due to increased production of propionate as higher propionate production results more conversion of propionate to methyl malonyl coA and further to succinyl Co-A which is intermediate metabolite of TCA cycle (Lean et al., 1992).

The feeding of a diet supplemented with monensin sodium observed significant treatment effect for plasma total protein concentration (P<0.05). A similar result was found in which total protein levels rose by around 1 g/dL, from 6.5 to 7.5 after monensin sodium feeding, indicating better amino acid absorption and liver efficiency (McGuffey et al., 2001). In present study, plasma cholesterol, triglycerides, albumin and globulin concentration remained statistically similar among treatments. Similar unaltered biochemical profile was stated by Oltenacu et al. (2023) in monensin-fed animals during lactation. Plasma cholesterol, triglycerides, total protein, albumin and globulin concentration remained statistically similar among treatments which follows the trend reported by Duffield et al. (2008) where serum protein and lipid status were unaffected by ionophore feeding in cows. Zahra et al. (2006) in a study found that monensin supplementation at 350 mg/day decreased free fatty acids concentrations by 20-25%, from 0.6 mEq/L to ~0.45 mEq/L. This indicates reduced lipolysis and improved energy balance. Mullins et al. (2012) also found a 20% reduction in liver triglycerides in cows given with 350 mg/day monensin.

Table 6. Effect of monensin sodium supplementation on blood biomarkers

Attributes	Supplemental Monensin Sodium (mg/animal/day)			SEM	Significance		
	Ms_0	Ms_{150}	MS_{300}		Treatment (T)	Period (P)	T×P
Glucose (mg/dL)	63.19[a]	68.03[a]	72.07[b]	1.61	0.028	0.501	0.346
Triacylglycerides (mg/dL)	25.97	26.55	23.78	0.74	0.744	0.942	0.921
Cholesterol (mg/dL)	146.00	144.27	146.11	1.16	0.784	0.080	0.997
Total protein (g/dL)	6.55[a]	6.69[a]	6.81[b]	0.06	0.047	0.022	0.009
Albumin (g/dL)	2.99	3.10	3.05	0.03	0.166	0.198	0.360
Globulin (g/dL)	3.36	3.47	3.72	0.07	0.131	0.590	0.302
BHBA (mmol/L)	0.77[a]	0.82[a]	0.52[b]	0.04	0.032	0.489	0.742
Insulin (mIU/L)	13.34	14.03	14.88	0.68	0.346	0.572	0.779
ALT (IU/L)	27.79	27.52	26.25	0.54	0.174	0.527	0.904
AST (IU/L)	91.70	84.83	87.45	1.50	0.437	0.752	0.932
ALP (IU/L)	90.92	79.32	83.46	2.38	0.435	0.832	0.986
Bilirubin (mg/dL)	0.40	0.44	0.41	0.00	0.756	0.184	0.173
PUN (mg/dL)	26.68	25.36	28.10	0.49	0.751	0.181	0.290
Creatinine (mg/dL)	1.12	1.14	1.07	0.01	0.534	0.134	0.186

BHBA=β-Hydroxy Butyric Acid; ALT=Alanine Aminotransferase; AST=Aspartat Aminotransferase; ALP=Alkaline Phosphatase; PUN=Plasma Urea Nitrogen. Means within a row with different superscript letters differ significantly (P<0.05).

In the present study, β-Hydroxy Butyric Acid (BHB) concentration was significantly lower in monensin-supplemented groups, especially at 300 mg/day, indicating improved energy balance and reduced ketone body accumulation. This finding is consistent with Duffield et al. (2008) who reported that monensin supplementation in transition cows reduced BHB levels and minimized the risk of subclinical ketosis. Similarly, McCarthy et al. (2013) observed a significant decline in circulating BHB and Non-esterified Fatty Acid (NEFA) values when monensin was fed to multiparous Holstein cows, reflecting greater glucose availability and reduced adipose mobilization. A comparable metabolic advantage was documented by Zeineldin et al. (2022) in early-lactation cows, where monensin administration improved ketone clearance and stabilized postpartum metabolic adaptation. The results of the present study therefore align with literature indicating that monensin promotes more efficient carbohydrate energy utilization, thereby decreasing reliance on fat-derived ketones. The reduction in BHB can be interpreted as a positive metabolic outcome as lower ketone levels reduce oxidative strain, support immune resilience and improve overall production energy efficiency in Sahiwal cows. Bovine insulin levels showed no significant variation among groups in the present study, indicating that monensin supplementation did not influence endocrine responses related to glucose metabolism. Comparable findings were reported by McGuffey et al. (2001), who observed stable insulin patterns in monensin-fed cattle. Similarly, Melendez et al. (2006) noted no alteration in insulin secretion in transition cows receiving monensin. Conversely, Grainger et al. (2010) documented reduced insulin concentrations under monensin supplementation.

Liver enzymes (ALT, AST, ALP and Bilirubin) and renal indicators (creatinine, PUN) remained within normal physiological range in all treatment groups, exhibiting no negative response to monensin supplementation. Similar observations were reported by Sanz-Fernandez et al. (2014) who supplemented monensin to multiparous dairy cows and noted no significant change in AST, ALT or creatinine despite improved metabolic efficiency. In agreement, Duffield et al. (2008) recorded normal liver and renal marker values in monensin-fed lactating cows, further confirming safety of the additive. In another controlled evaluation on early-lactation cows, O'Connor et al. (2018) also reported non-significant changes in ALT, AST and PUN upon monensin inclusion findings nearly identical to our results. The consistency across multiple independent studies strengthens the interpretation that monensin does not impose hepatic or renal stress when fed within recommended dosage, thus validating its metabolic safety in lactating Sahiwal cows.

Conclusion

The present study concludes that monensin sodium supplementation in lactating Sahiwal cattle did not affect dry matter intake, body weight gain, milk composition, most of blood biomarkers, mineral status, or overall health. However, improved organic matter and crude protein digestibility, increased milk yield, higher plasma glucose, total protein and reduced BHBA concentration particularly at the higher supplementation level indicate enhanced rumen fermentation and energy metabolism. Thus, monensin sodium supplementation appears to be a safe and effective nutritional strategy for improving milk production in lactating Sahiwal cattle without compromising animal health or milk quality.

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Statements & Declarations:

Ethical requirements & declaration: The experiment was carried out at the Livestock Farm Complex (LFC), DUVASU, Mathura. Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC), DUVASU, Mathura (Approval No. IAEC/25/59), constituted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Data Availability statement: None of the data were deposited in an official repository and are available upon request.

AI policy statement: The authors carefully reviewed, revised, and verified all AI-generated content to ensure its accuracy, scientific integrity, and compliance with journal standards. The authors take full responsibility for the content of the manuscript, including all interpretations, analyses, conclusions, and any errors that may remain. No AI tool was involved in the generation, analysis, or interpretation of the research data unless explicitly stated elsewhere in the manuscript.

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Authors contribution: Dhapse Sanket Rajkumar carried out animal trial, sample analysis, and wrote the first draft of the manuscript. Raju Kushwaha conceptualized and designed this study, conducted data analysis, and wrote the final draft of the manuscript. Vinod Kumar and Muneendra Kumar supervised the overall study. Shalini Vaswani and Avinash Kumar reviewed the manuscript. Ram Dev Yadav and Mokshata Gupta edited the final manuscript. Kajal Rajvanshi helped during the animal trial and sample analysis.

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