

Impact of Feeding Ration Partially Replaced by Rice DDGS on Reproductive Hormonal Profile and Performance in Gir Heifers

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ABSTRACT

The effects of partial substituting rice distillers dried grains with solubles (RDDGS) for concentrate protein in the diet of 24 pre-pubertal/pubertal Gir heifers (average b.wt. 166.29 ± 11.21 kg, age 830.62 ± 13.67 days) were studied over 180 days for reproductive hormonal profile and performance at University farm, Junagadh. The animals were randomly divided into three equal groups, each consisting of 8 heifers, viz., Control (100% crude protein (CP) from compounded concentrate mixture), T-1 (25% CP replaced with RDDGS) and T-2 (50% CP replaced with RDDGS). The basal diet of 10 kg seasonal green, dry fodder *ad libitum* and a mineral mixture @ 30 gm/h/d was given to the experimental heifers in addition to their daily and individual treatment diets. Heifers were fed a diet based on ICAR (2013) standards and the reproductive hormone levels in blood were measured on days 0, 90 and 180. The experimental heifers were daily observed for signs of estrus. Development and functioning of reproductive system of Gir heifers was assessed per-rectally at monthly intervals. Results showed that RDDGS increased progesterone levels, while estrogen remained unchanged. 75% of RDDGS-fed heifers exhibited earlier signs of puberty, with better reproductive tract development compared to the control group. Therefore, replacing 25% concentrate protein with RDDGS can be recommended for earlier puberty and improved reproductive performance in Gir heifers.

Key words: DDGS, Estrus, Gir heifer, Ovarian structure, Reproductive hormones.

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INTRODUCTION

Animal feed is vital for the growth, productivity and reproduction of dairy cattle in India, making up 60-70% of production costs. Efficient feed management enhances growth, production, reproduction, feed efficiency and farm economics, reducing expenses and maximizing profitability. Principal obstacles are rising feed costs, restricted availability and shrinking acreage for fodder. Alternative feed choices have gained popularity due to the high costs and seasonal availability of traditional protein sources such as soybean and groundnut cake (Chatterjee *et al.*, 2016).

Rice distillers dried grains with soluble (RDDGS), an agro-industrial by-product from the alcohol and ethanol industries in Asia, offers ruminants energy and high levels of rumen undegradable protein (RUP). Due to its high protein, good amino acid profile and affordability, RDDGS is a valuable feed option comparable to other fermented by-products, with added benefits from its high fibre and mineral content (Chatterjee *et al.*, 2016). Due to high oil content with more of polyunsaturated fatty acids in DDGS, feeding larger amounts of DDGS increase dietary fat in addition to dietary crude protein. This could affect the results of reproduction as well as performance (Gunn *et al.*, 2015). Optimizing heifer growth through economical feeding is necessary to reduce the age at puberty and calving, which increases economic returns. Distillers dried grains (DDG) supplementation

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during development of beef heifers had no effect on the onset of puberty, but improved reproductive performance by reducing number of AIs per conception and increasing pregnancy rates (Martin *et al.*, 2007). Despite its nutritional value, DDGS's effects on native breeds like Gir cattle are not well understood, as most research focuses on exotic breeds. To address this, a study was conducted to evaluate the reproductive performance and hormone profile of pre-pubertal/puberal Gir heifers fed diets partially replaced with DDGS.

MATERIALS AND METHODS

The study was conducted following approval of IAEC (Protocol No. KU-JVC-IAEC-LA-106-23) at Cattle Breeding Farm, Kamdhenu University, Junagadh (Gujarat, India). Twenty-four prepubertal/puberal Gir heifers, averaging 27 months of age (830.62 ± 13.67 days), were monitored for 6 months after a 7-day adaptation period to DDGS supplementation. The heifers were randomly divided into three equal groups, each of 8 animals (Control, T-1, T-2) for feeding trials. The heifers of Control group received 100% CP from compounded concentrate mixture, while those in T-1 and T-2 groups diet, 25% and 50% CP was replaced with rice DDGS, respectively. The basal diet of 10 kg seasonal green, dry fodder *ad libitum* and a mineral mixture @ 30 gm/h/d was given to the experimental heifers in addition to their daily and individual treatment diets to meet the nutrients requirements as per ICAR (2013) standards, with ration adjustments every 15 days. They had unlimited access to clean water and were monitored for signs of heat, with 2 h of roaming daily.

Representative feed and fodder samples and RDDGS were tested for proximate principles (AOAC, 2023). The blood samples were collected for biochemical parameters in heparinized vacutainers at days 0, 90th and 180th of the experiment. The serum was separated by centrifugation of blood samples at 1265 g for 20 min and stored properly in plastic storage vials at -20°C in deep freezer until analysis. The serum hormone progesterone and estrogen profiles of Gir heifers were measured at days 0, 90 and 180 using ELISA kits (Genetix Biotech Asia Pvt. Ltd., Batch No. PG-0018Bo and PG-0240Bo). Monthly per-rectal examinations assessed the growth and functionality of their genitalia and ovaries.

All experimental heifers were observed twice daily for signs of heat. The data on reproductive behaviour found was analysed by descriptive statistics and those on hormone profile by using one way ANOVA and Duncan's post hoc multiple range test on SPSS software (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Proximate Composition of Feed Stuff

The percent dry matter and organic matter found on DM basis in different feed stuffs used in the study as well as proximate component it contained are shown in Table 1. Data show that the crude protein content was the highest in rice DDGS followed by concentrate mixture, groundnut gotar, and green fodder, while crude fibre showed reverse trend, and ether extract and total ash content of rice DDGS were quite low compared to other feed stuffs, suggesting its higher nutritional value.

Reproductive Hormonal Profile

The serum progesterone levels (Table 2) showed significant variation at day 90, but not at days 0 or 180, with T-1 having the highest levels in the experimental Gir heifers. On day 0 progesterone values were almost similar in all 3 groups (<1.0 ng/ml) suggesting that all the animals were practically non-cyclic at this age or stage. However the values on day 90 and overall average levels of progesterone throughout the study period showed significant differences between groups. The T-1 group had the highest overall level (1.81 ± 0.17 ng/mL), followed by T-2 (1.65 ± 0.13 ng/mL) and the Control group had the lowest (1.13 ± 0.09 ng/mL). On day 180 also the trend was more or less similar with higher serum progesterone concentration in RDDGS fed groups, though statistically non-significant. This happens due to cyclic nature of animals and variable stage of estrous cycle on the day of sampling. The findings clearly showed that by 90 days initiating RDDGS feeding some animals resume ovarian cyclicity with ovulation and formation of corpus luteum on the ovaries. The high fat content in distillers dried grains with solubles (DDGS) may also affect progesterone production by influencing the availability of cholesterol, the precursor molecule for

Table 1: Proximate composition of seasonal green fodder, dry fodder and compound concentrate mixture and rice DDGS (% DM basis)

Proximate components	Green sorghum	Napier grass	Groundnut gotar	Compounded conc. mix.	Rice DDGS
Dry matter (DM)	23.20	22.70	90.43	90.52	90.28
Organic matter (OM)	91.55	88.13	88.39	87.27	95.53
Crude protein (CP)	06.70	06.90	10.40	20.15	40.12
Crude fibre (CF)	31.25	27.18	29.37	10.24	04.98
Ether extract (EE)	03.10	03.35	04.25	02.76	06.02
Nitrogen free extract (NFE)	51.70	44.37	32.37	54.12	44.41
Total ash	10.87	11.61	23.61	12.73	04.47

steroid hormone synthesis, including progesterone. These results were consistent with those of Anderson *et al.* (2015), who showed elevated progesterone levels in high-fat DDGS diet-fed Holstein heifers. However, no discernible effects of DDGS were observed on progesterone in crossbred heifers or cows by Subramaniam *et al.* (2016) and Dhama *et al.* (2021). Nevertheless, Kennedy *et al.* (2016) observed that the combination of treatment and days of feeding ($p < 0.01$) led to a consistent rise in progesterone levels in cows fed diet without DDGS.

On days 0, 90, and overall, the mean serum estrogen concentrations were found to be identical, of course with little rise in levels with increase in age and feeding time, but by 180th day of the experiment, the mean estrogen levels showed a significant difference ($p < 0.01$) between the groups with further rise ($p < 0.05$) in levels over day zero. The T-2 group had the highest estrogen level (26.25 pg/mL), followed by T-1 (23.75 pg/mL) and the Control group had the lowest (22.50 pg/mL) mean value, while there were no significant differences at other time points (Table 2). The current results corroborated with those of Dhama *et al.* (2021) in crossbred heifers (22.42 ± 1.50 vs. 22.26 ± 1.41 vs. 22.24 ± 1.83 pg/mL; $p > 0.05$) and they observed it fluctuating as per the ovarian cycle in different groups at different time intervals reflecting presence of anovulatory follicles in some of the animals. Subramaniam *et al.* (2016) in cows also showed that plasma estrogen content did not vary across feeding groups. The estrogen-progesterone concentration is influenced not only due to the effect of feeding, but it also increased with advancing experimental period or age, which is a physiological phenomenon in pubertal/post-pubertal heifers.

Reproductive Performance of Experimental Groups

Experimental Gir heifers were monitored for serum hormonal profiles and observed for estrus signs twice daily, with reproductive performance evaluated monthly via per-rectal exams. Majority of heifers (75%) fed with distillers dried grains with solubles (DDGS) showed estrus signs, like vaginal

discharge, 2 to 3 months earlier than the control group, where only 50% heifers exhibited signs by the 5th month. More numbers of heifers repeatedly displayed sexual heat during the period of the experiment in the RDDGS fed groups, but were not bred (Table 3). This earlier onset of estrus in DDGS-fed heifers is linked to its high nutritional content, including energy, protein, vitamins and minerals.

During the experimental period, Gir heifers fed with distillers dried grains with solubles (DDGS) showed superior reproductive development compared to the control group. The DDGS-fed heifers exhibited good uterine development, with palpable follicular development on the ovaries and the presence of the corpus luteum (CL), along with mild to moderate uterine tone. The presence of developed follicles indicates that these heifers were approaching the ovulation phase, suggesting that their hormonal systems were functioning well, which is crucial for releasing mature oocytes and ensuring successful fertilization and pregnancy. The formation of the corpus luteum (CL) after ovulation is significant, as it secretes progesterone, a hormone necessary to maintain pregnancy, if fertilization occurs. The mild to moderate uterine tone observed in DDGS-fed heifers indicates a balanced uterine state, neither overly relaxed nor excessively tense, which reflects normal physiological function and readiness for reproduction. These observations suggest that the nutritional profile of DDGS, rich in energy, protein, vitamins and minerals, positively impacts reproductive health and performance.

In this study, partially replacing compound concentrate mixture with DDGS improved reproductive performance in Gir heifers. However, Engel *et al.* (2008) found no effect on estrus initiation ($p = 0.46$). Damiran *et al.* (2016) and Taylor *et al.* (2017) also reported no significant impact on cow reproductive performance and in cyclicity resumption or follicle size in heifers, respectively. Manthey *et al.* (2017) noted that 50% DDGS-fed Holstein heifers began cycling at a younger age. Dhama *et al.* (2021) observed ovarian activity

Table 2: Mean \pm SE values for serum progesterone and serum estrogen in experimental Gir heifers during experimental period

Serum hormone	Days of feeding	Treatment groups			P-value
		Control (n=8)	T-1 (n=8)	T-2 (n=8)	
Progesterone (ng/mL)	0	0.92 \pm 0.23	0.90 \pm 0.15	0.93 \pm 0.15	NS
	90 th	0.92 ^c \pm 0.15	2.09 ^a \pm 0.11	1.59 ^b \pm 0.23	0.0003
	180 th	1.55 \pm 0.29	2.42 \pm 0.49	2.43 \pm 0.26	NS
	Overall	1.13 ^b \pm 0.09	1.81 ^a \pm 0.17	1.65 ^a \pm 0.13	0.006
Estrogen (pg/mL)	0	17.00 \pm 1.70	15.25 \pm 2.20	16.88 \pm 1.17	NS
	90 th	19.12 \pm 1.29	21.38 \pm 0.68	20.88 \pm 0.39	NS
	180 th	22.50 ^c \pm 0.42	23.75 ^b \pm 0.36	26.25 ^a \pm 0.41	0.0001
	Overall	19.54 \pm 0.87	20.12 \pm 0.74	21.33 \pm 0.50	NS

C: 100% crude protein (CP) from compound concentrate mixture; T-1: 75% CP from compound concentrate mixture + 25 % CP from rice DDGS; T-2: 50% CP from compound concentrate mixture + 50 % CP from rice DDGS. Means in a row with dissimilar superscripts differ significantly ($p < 0.01$)



Table 3: Observed estrus during experiment of pre-pubertal / pubertal Gir heifers

Group	No. (%) exhibited sexual heat	Observed estrus during month of experiment*						Total
		1	2	3	4	5	6	
Control	04 (50%)	0	0	1	1	2	2	6
T-1	06 (75%)	0	2	3	4	4	4	17
T-2	06 (75%)	0	1	2	3	4	4	14

*Figures represent number of heifers with repeated sexual cycles in a particular month, Control: 100% crude protein (CP) from compound concentrate mixture; T-1 & T-2, 25 % & 50% CP replaced by Rice DDGS, resp.

in crossbred heifers starting at 14 months, with 60% pregnant by 19 months and 100% by 23 months when rice DDGS replaced soya cake in the diet.

CONCLUSION

During the present rice DDGS feeding trials of 180 days, vaginal discharge was seen in 75% of the heifers fed DDGS by 2 to 3 months earlier than in the control group. Throughout the experiment from 2nd month onwards, the DDGS-fed groups also exhibited mild to moderate uterine tone, greater ovarian follicle growth, corpus luteum (CL) and higher progesterone levels. Gir heifers that had part of their diet replaced with DDGS had better onset of reproductive cycles and a normal hormonal profile without any adverse effect of their health status. Hence, replacing 25% concentrate protein with RDDGS can be recommended for earlier puberty and improved reproductive performance in Gir heifers.

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