

Evaluation of distillers dried grains with solubles (DDGS) as an alternative protein source in adult dog food

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Abstract

The present study aimed to achieve two main objectives. First, an in vitro evaluation of the digestibility of dried distillers' grains with solubles (DDGS) was performed to establish optimal inclusion levels in adult dog diets. Diets containing 0%, 5%, 10%, 15%, and 20% DDGS were formulated by replacing soybean meal based on ICAR (2013) nutrient recommendations. The results showed that dry matter, organic matter, crude protein, and ether extract digestibility were significantly higher ($p < 0.05$) in diets with up to 15% DDGS compared to the 20% DDGS diet. Consequently, the 10% and 15% DDGS diets were selected for further evaluation. In the second phase of the study, twelve healthy adult Beagle dogs (36–40 months old; average body weight: 12.13 ± 0.29 kg) were randomly assigned to three dietary groups (0%, 10%, or 15% DDGS) for a 90-day period. There were no significant differences ($p > 0.05$) in dry matter intake or changes in body weight among the groups. However, nutrient digestibility of dry matter, organic matter, crude protein, and ether extract was significantly greater ($p < 0.05$) in the 0% and 10% DDGS groups compared to the 15% group, while crude fiber digestibility remained comparable across all groups. Blood and serum biochemical indices, including hemoglobin, packed cell volume, glucose, total protein, albumin, SGOT, SGPT, blood urea nitrogen, creatinine, total cholesterol, and triglycerides, showed no significant differences ($p > 0.05$). Similarly, gut health indicators (fecal pH, lactic acid, ammonia) and the cell-mediated immune response to phytohemagglutinin-P (PHA-P) were not affected by DDGS inclusion. In conclusion, DDGS is a suitable alternative protein source in adult dog diets and can be included at levels of up to 10% without compromising nutrient digestibility, health, or immune response.

Keywords: Adult dog; blood-biochemical; DDGS; digestibility; gut health; immunity

Introduction

Pet adoption rates have risen significantly in India, leading to a dramatic increase in the pet population from 18.1 million in 2016 to 31.7 million in 2021 reflecting a compound annual growth rate (CAGR) of 11.9%. This number is projected to reach 50.1 million by 2026, with an expected CAGR of 18.5%, of which approximately 90% are dogs. Alongside this growth, the pet food market has expanded, reaching USD 403.4 million in 2021 (CAGR of 9.6%), and is anticipated to grow to USD 887.6 million by 2026, with a CAGR of 17.1% (Bernardi, 2023). Similar to the livestock and poultry feed industries, the dog feed industry primarily depends on conventional feed resources, which form a major part of commercial pet diets. However, the availability of these feed ingredients is often limited, subject to price fluctuations, and competes with the livestock and poultry sectors posing major challenges to the growth and sustainability of the pet food industry. To address these issues, animal nutritionists are exploring unconventional feed ingredients that are both cost-effective and capable of supporting optimal health and performance in companion animals. One such promising feed ingredient is dried distillers' grains with solubles (DDGS), a byproduct of the ethanol industry obtained from the fermentation of cereal grains (Sharyari et al., 2020; Ayandiran et al., 2023). DDGS is recognized as a rich source of protein, methionine, fat, and minerals particularly phosphorus (Gupta et al., 2020). Global DDGS production is on the rise due to increasing demand for biofuels. In India, the target of blending 20% ethanol with petrol by 2025 is expected to significantly enhance DDGS availability. This surplus presents an opportunity for DDGS to be utilized as an economical and sustainable protein source in the diets of livestock, poultry, and pets. In poultry and swine nutrition, partial replacement of soybean meal with DDGS has shown no adverse effects on production performance [Gupta et al., 2020; Risolia et al., 2019]. However, limited studies have investigated the use of DDGS in canine diets as a substitute for conventional protein sources (Khose et al., 2018; Silva et al., 2016), and a significant knowledge gap remains regarding its potential as an alternative to soybean meal for dogs. Therefore, the present study was designed with two primary objectives: (1) to evaluate the *in vitro* digestibility of DDGS in adult dog diets to determine optimal inclusion levels, and (2) to assess the effects of DDGS inclusion, as a replacement for soybean meal, on nutrient digestibility, blood biochemical parameters, immune response, and gut health in adult dogs.

Materials and methods

Study location

The study was conducted during the summer season at the Department of Animal Nutrition and the Dog House Facility, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab, India-141001. The Department of Animal Nutrition is geographically located at 30.9021° N latitude and 75.8046° E longitude, whereas the Dog House Facility is situated at 30.8947° N latitude and 75.8070° E longitude. The *in vitro* analyses, including nutrient digestibility assessments and laboratory investigations, were carried out in the Department of Animal Nutrition. The *in vivo* feeding trial and sample collection were conducted at the Dog House Facility.

Proximate analysis of DDGS and different adult dog diets

The dried distillers' grains with solubles (DDGS) used in the present study were procured from BCL Industries Limited, Bathinda, Punjab, India. The procurement price of DDGS at the time of the experiment was ₹30/kg. Samples of DDGS, the individual feed ingredients, and the experimental adult dog diets containing 0, 5, 10, 15, and 20% DDGS were analysed for their proximate composition and nutrient profile using standard analytical procedures.

Formulation of Dog Diets

Dog diets were formulated based on the proximate analysis findings using MS Excel. A thorough evaluation of various feed ingredients guided the development of diets containing specific levels of key nutrients. As per (ICAR, 2013), the diets were designed to provide 18% crude protein (CP), 9% ether extract (EE), 0.5% calcium (Ca), and 0.4% phosphorus (P). Additionally, the energy density of the diets was set at 3,500 kcal ME/kg.

Extrusion of diets

For the extrusion (Singh et al., 2022) of the experimental diets, the finely ground food ingredients and DDGS were mixed in the required proportions (Table 1) and stored in vats. A die corresponding to the desired pellet shape and size was fitted to the front of the extruder. The twin-screw extruder operated at a barrel speed of 35 Hz and was equipped with three heaters set at different temperatures: Heater I at 80°C, Heater II at 120°C, and Heater III at 160°C.

In vitro digestibility of the experimental diets

The *in vitro* assessment of dog diets incorporating DDGS at various concentrations (0, 5, 10, 15, and 20%) followed the protocol outlined by (Biagi et al., 2016), comprising two incubation phases. The first phase involved a 2-hour gastric incubation with pepsin, gastric lipase, and HCl, followed by a 4-hour intestinal phase using a buffer solution containing pancreatin and bile salts. After incubation, the undigested food samples were filtered through nylon bags, washed with cold water, and dried in a hot air oven at 65°C until a constant weight

was achieved. The dry weight of each nylon bag was then recorded, and the remaining content was preserved for subsequent analysis. The in vitro digestibility trial was conducted in triplicate for each treatment.

Calculation and data analysis

The residual material retrieved from each container after in vitro digestion was weighed, and the in vitro dry digestibility of the digested feed residue was calculated via the provided equation.

$$\text{In vitro Dry matter digestibility} = 100 - \frac{\text{Residue weight} \times 100}{\text{Sample weight}}$$

The undigested fraction was analysed for OM, CP, and EE.

$$\text{Nutrient Digestibility} = 100 - \frac{\text{Nutrient \% in residue} \times (100 - \text{diet digestibility})}{\text{Nutrient \% in diet}}$$

Aflatoxin estimation of the diets

Aflatoxin estimation was performed using an AflaTest® fluorometer (VICAM 4EX Series, Waters, Milford, MA, USA), with a detection range of 0–500 ppb and a detection limit of 2 ppb. For sample extraction, 50 g of food was blended with a methanol:water (80:20) mixture, filtered, and appropriately diluted. Column chromatography was carried out by passing the filtered extract through an AflaTest® column (Waters, Milford, MA, USA), followed by the addition of a developer solution (Waters, Milford, MA, USA). The resulting solution was then analyzed using the fluorometer to detect the presence of aflatoxins.

Experimental animals

All animals used in the trial were healthy, active, and responsive to human presence. A total of twelve Beagle dogs, aged between 36 and 40 months with an average body weight of 12.13 ± 0.29 kg, were randomly assigned to three groups of four animals each using a completely randomized design, ensuring equal distribution of sexes across the groups. For exercise, the dogs were allowed outside for one hour twice daily in a large area adjacent to the kennel. Every two weeks, the animals were bathed using a benzoyl peroxide-containing shampoo. All dogs were dewormed with Drontal Plus (containing praziquantel 50 mg, pyrantel embonate 144 mg, and febantel 150 mg per tablet) at a dose rate of one tablet per 10 kg body weight. Prior to the commencement of the experiment, the dogs were vaccinated against various contagious diseases, including distemper, hepatitis, leptospirosis, parvovirus, parainfluenza, and rabies.

Housing and environment

All dogs were housed in the experimental dog facility of the institute, which is duly approved by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India, New Delhi. The experimental protocol was sanctioned by the Institutional Animal Ethics Committee (IAEC) of the university, bearing the protocol number GADVASU/2023/IAEC/69/16. Each experimental dog was housed individually in a cage (5'×3'×4') within an environmentally controlled facility. The kennel was maintained at optimal conditions for canine comfort, including temperature (18–24°C), illumination (12-hour light/dark cycle), and relative humidity (37%–70%). To ensure hygiene, the facility was cleaned twice daily, covering both individual cages and the surrounding open areas. The dogs were regularly exposed to animal attendants and researchers to facilitate acclimatization to the experimental environment and handling procedures. Throughout the study, the health of the animals was closely monitored, including their general physical condition, changes in body weight, coat quality, and any signs of illness. Each dog was identified using metallic tags attached to collars, according to its treatment group.

Dietary treatments

Based on the in vitro digestibility study, the two best-performing DDGS inclusion levels (10% and 15%) were selected. Group I received a basal diet containing 0% DDGS (soybean meal-based), Group II received a basal diet with 10% DDGS, and Group III was fed a diet containing 15% DDGS. All diets were formulated according to the feeding standards described in ICAR, 2013, and the experimental feeding period lasted 90 days.

Body weight changes and nutrient digestibility

Fortnightly body weight changes were recorded by measuring body weight via an electronic weigh balance every two weeks at weekly intervals in the morning before providing feed and water throughout the experimental duration of 90 days. To determine the digestibility of nutrients, a four-day digestion trial was conducted at the end of the experiment. After the total amount was measured, representative samples of feed, residue, and feces were pooled animal wise for one day and preserved for analysis. The dried pooled samples of feed offered, residue left, and feces were analysed for proximate principles as per [8], and digestibility was calculated via mathematical formulas.

$$\% \text{ Digestibility} = \frac{\text{amount of nutrient consumed} - \text{amount of nutrient excreted in faeces}}{\text{amount of nutrient consumed}} \times 100$$

Palatability score

Palatability of the experimental dog foods was assessed during the digestion trial using a 4-point subjective scoring scale

Fecal score

Dog feces were collected daily and evaluated using a 5-point subjective scoring system

Blood biochemical profile

Blood samples (4 mL) were collected from each dog on days 0 and 90 of the experimental trial. A portion of each sample was transferred into EDTA vials for hematological analysis, while the remaining portion was collected in serum activator vials for serum biochemical analysis. Serum samples were analyzed for various biochemical constituents using commercial diagnostic kits and an automated clinical chemistry analyzer (Vitros® 350 Chemistry System, Ortho-Clinical Diagnostics Inc., Johnson & Johnson, SA).

Cell-mediated immune response

To assess the *in vivo* cell-mediated immune response, a type IV delayed-type hypersensitivity (DTH) reaction was induced. On the 86th day of experimental feeding, all animals were intradermally injected with 100 µL of phytohemagglutinin-P (PHA-P) mitogen (0.5 mg/mL in saline solution). The injection site was clipped and disinfected with 70% ethanol. Skin induration at the injection site was measured at 0, 12-, 24-, 48-, and 72-hours post-injection using a digital Vernier caliper. The DTH response was expressed as the percentage increase in skin thickness relative to the 0-hour measurement.

Gut health parameters

To evaluate the effect of DDGS inclusion on gut health, parameters such as fecal pH, fecal ammonia, and fecal lactic acid concentrations were measured. Fecal pH was determined using a Systronics® µpH System 361, calibrated with standard buffer solutions (pH 4.0, 7.0, and 9.2). After calibration, the electrode was immersed in freshly voided, homogenized feces from individual dogs, and readings were recorded.

Statistical analysis

The data generated from all experimental parameters were statistically analyzed using IBM SPSS software (version 16). A generalized linear model (GLM) ANOVA procedure was employed to compare treatment groups. Duncan's multiple range test was used for post hoc analysis to determine significant differences among means. Differences were considered statistically significant at $p < 0.05$.

Results**Chemical Composition of DDGS and Experimental Diets**

The chemical composition of DDGS and the various experimental diets is presented in Tables 2 and 3. Analysis of DDGS revealed the following composition: dry matter (DM) 93.1%, crude protein (CP) 41.98%, ether extract (EE) 2.0%, crude fiber (CF) 12.03%, total ash 5.17%, acid-insoluble ash (AIA) 1.36%, calcium (Ca) 0.06%, and phosphorus (P) 0.2%. The experimental extruded diets, prepared with different DDGS inclusion levels (0%, 5%, 10%, 15%, and 20%), were isocaloric and isonitrogenous.

In Vitro Digestibility Study

The *in vitro* digestibility results (Table 4) showed that the digestibility of DM, organic matter (OM), EE, and CP was significantly higher ($p < 0.05$) in the 0%, 5%, 10%, and 15% DDGS diets compared to the 20% DDGS diet. Based on these findings, the 10% and 15% DDGS inclusion levels were selected for the *in vivo* study in adult Beagle dogs.

Aflatoxin Levels in DDGS and Experimental Diets

The total aflatoxin levels in DDGS and the selected diets (0%, 10%, and 15% DDGS) were 16 ppb, 4 ppb, 6.6 ppb, and 7.3 ppb, respectively.

Palatability Score

All dogs across the dietary treatments consumed their meals quickly, resulting in a palatability score of 1, indicating strong acceptance and palatability of all experimental diets.

Nutrient Digestibility

As shown in Table 5, the digestibility of DM, OM, CP, and EE was significantly higher ($p < 0.05$) in the 0% and 10% DDGS groups compared to the 15% DDGS group. However, the digestibility of CF was statistically similar ($p > 0.05$) across all treatments.

Blood Biochemical Parameters

Blood biochemical parameters (Table 6) including hemoglobin (Hb), packed cell volume (PCV), glucose, total protein, albumin, creatinine, blood urea nitrogen (BUN), SGPT, SGOT, total cholesterol, and triglycerides showed no significant differences ($p > 0.05$) among the groups. All values remained within the normal physiological range, indicating that the dogs remained healthy throughout the experiment.

Fecal Score

Fecal scores for dogs fed 0%, 10%, and 15% DDGS-based diets were 4.06, 4.31, and 3.81, respectively. These differences were not statistically significant ($p > 0.05$), indicating consistent stool quality across treatments.

Table 1: Physical composition of diets with different levels of DDGS

Ingredients (kg)	0 % DDGS	5%DDGS	10%DDGS	15%DDGS	20%DDGS
Maize grain	38	38	38	38	38
Soybean meal	10	5	0	0	0
DDGS	0	5	10	15	20
Rice gluten meal	4.75	6.5	8.5	5	2.25
Black gram	11	9.25	7.25	5.75	3
Milk powder	3	3	3	3	3
Rice grain	22	22	22	22	22
Vegetable oil	7.5	7.5	7.5	7.5	8
Meat cum bone meal	3	3	3	3	3
Feed additives*	0.75	0.75	0.75	0.75	0.75

*Trace minerals (0.15%), vitamin premix (0.015%), toxin binder (0.05%), yeast (0.2%), acidifier (0.2%), vitamin B12 (0.025%), emulsifier (0.025%), BHT (0.05%), enzymes (0.025%), B complex vitamins (0.01%); DDGS: Dried distillers' grains with solubles

Table 2: Chemical composition of dried distillers' grains with solubles (DDGS)

Parameters	Level
Dry matter	93.1 %
Crude protein	41.98%
Ether extract	2.0%
Crude fibre	12.03%
Calcium	0.06%
Phosphorus	0.2%
Ash	5.17%
Acid insoluble ash	1.36%

Table 3: Chemical composition of extruded dog food prepared from graded levels of DDGS

Parameters (%)	0% DDGS	5% DDGS	10% DDGS	15% DDGS	20% DDGS
Dry Matter	96.59	96.57	96.75	96.87	96.83
Crude Protein	19.14	19.55	19.56	19.25	19.51
Ether Extract	7.71	7.95	7.81	7.91	7.83
Crude Fibre	3.57	3.69	3.73	3.75	3.95
Calcium	0.56	0.56	0.56	0.53	0.51
Phosphorus	0.50	0.51	0.49	0.50	0.48
Total Ash	3.44	3.97	3.58	3.35	3.86
Acid insoluble ash	0.59	0.61	0.64	0.61	0.65
GE (kcal/kg)	4607	4646	4774	4758	4738
ME*(Kcal/kg)	3521	3509	3521	3537	3507

ME: calculated by modified Atwater physiological fuel value (NRC, 2006); DDGS: Dried distillers' grains with solubles

Table 4: *In vitro* digestibility of dog food with different levels of DDGS

Digestibility%	Dietary treatments ¹					SEM ¹	P Value ²	Contrast ³	
	% DDGS	5% DDGS	10% DDGS	15% DDGS	20% DDGS			Linear	Quadratic
Dry matter	86.80 ^a	86.21 ^a	85.93 ^a	85.78 ^a	81.87 ^b	0.490	<0.001	<0.001	0.001
Organic matter	87.15 ^s	86.69 ^a	86.35 ^a	86.03 ^a	82.05 ^b	0.527	<0.001	<0.001	0.004
Ether extract	92.88 ^a	92.35 ^a	92.09 ^a	91.86 ^a	86.06 ^b	0.793	0.008	0.003	0.03
Crude protein	90.40 ^a	90.08 ^a	89.44 ^a	88.89 ^a	80.57 ^b	1.075	0.001	<0.001	0.004

^{ab} means bearing different superscripts within a column differ significantly (p<0.05); DDGS: Dried distillers' grains with solubles

Gut Health Parameters

Gut health parameters (Table 6) including fecal pH, fecal ammonia, and fecal lactic acid concentrations, showed no significant differences (p>0.05) among the treatment groups, suggesting that DDGS inclusion did not adversely affect gut health.

Cell-Mediated Immune Response

Cell-mediated immune response, assessed by the delayed-type hypersensitivity (DTH) reaction to phytohaemagglutinin-P (PHA-P) and expressed as the increase in skin thickness at 12, 24, 48, 72, and 96 h post-injection, did not differ significantly (p>0.05) among the dietary groups. The mean skin thickness was 9.99, 9.92, and 10.90 mm in the control, 5% DDGS, and 10% DDGS groups, respectively, indicating that dietary inclusion of DDGS up to 10% did not adversely affect cell-mediated immunity in adult Beagle dogs.

Table 5: Dry matter intake and percent Apparent total tract digestibility of nutrients and body weight changes in dog fed different levels of DDGS

Digestibility%	Dietary treatments ¹			SEM ¹	P Value ²	Contrast ³	
	0% DDGS	5% DDGS	10% DDGS			Linear	Quadratic
Dry matter intake (g/day)	326.84	311.74	313.68	5.753	0.522	0.391	0.517
Apparent total tract digestibility (%)							
Dry matter	85.31 ^a	84.53 ^a	78.49 ^b	1.057	0.02	0.001	0.061
Organic matter	87.00 ^a	86.16 ^a	80.93 ^b	0.942	0.02	0.001	0.084
Ether extract	93.53 ^a	92.41 ^a	87.07 ^b	0.897	<0.001	<0.001	0.02
Crude protein	85.68 ^a	84.26 ^a	76.74 ^b	1.318	0.001	<0.001	0.052
Crude fibre	38.66	38.27	37.60	3.008	0.993	0.906	0.988
Body weight changes (kg)							
Initial body weight	12.37	12.05	11.96	0.289	0.860	-	-
Final body weight	12.59	12.30	12.21	0.288	0.873	-	-
Net change	0.22	0.25	0.24	0.007	0.404	-	-

^{ab} means bearing different superscripts within a column differ significantly ($p < 0.05$); DDGS: Dried distillers' grains with solubles

Table 6: Blood biochemical and gut health parameters of adult dogs fed different levels of DDGS

Parameters	Dietary treatments ¹			SEM ¹	P Value ²	Contrast ³	
	0% DDGS	5% DDGS	10% DDGS			Linear	Quadratic
Hb (g/dl)	13.43	13.35	13.38	0.685	0.990	0.928	0.916
PCV (%)	43.30	42.39	42.21	3.097	0.888	0.659	0.863
Glucose (mg/dl)	100.75	102.25	104.00	7.062	0.828	0.563	0.979
Total protein (g/dl)	6.75	6.70	6.45	0.239	0.165	0.081	0.469
Albumin (g/dl)	3.38	3.53	3.48	0.337	0.843	0.708	0.666
SGOT (U/l)	31.50	31.00	30.00	5.060	0.928	0.711	0.943
SGPT (U/l)	35.75	36.25	35.50	1.801	0.861	0.861	0.615
BUN (mg/dl)	8.48	8.78	8.48	0.273	0.212	1.000	0.086
Creatinine (mg/dl)	0.61	0.50	0.47	0.158	0.489	0.266	0.713
Total cholesterol (mg/dl)	150.75	152.75	154.75	7.362	0.780	0.493	1.000
Triglycerides (mg/dl)	52.50	58.75	57.00	16.071	0.875	0.725	0.718
Faecal pH	5.60	5.69	5.76	0.050	0.448	0.218	0.930
Faecal Ammonia (μmol/g DM faeces)	40.34	41.38	41.18	0.535	0.739	0.564	0.619
Faecal Lactic Acid (μmol/g DM faeces)	25.98	28.10	26.80	0.858	0.636	0.809	0.392

DDGS: Dried distillers' grains with solubles

Discussion

Chemical Composition of DDGS and Experimental Diets

Chemical analysis revealed that DDGS is a valuable alternative protein source, with a crude protein (CP) content of 41.98%. Similarly, (Kumar et al. 2023) reported that rice DDGS contained 45% CP, 27.12% ether extract (EE), and 4.89% crude fiber (CF), with a metabolizable energy (ME) value of 2880 kcal/kg. (Kaninde et al., 2023) found that DDGS contained 40.17% CP, 5.33% CF, 7.67% EE, 5.06% total ash, 0.35% calcium, and 0.71% phosphorus. Additionally, the CP content of DDGS has been reported as 48.10% by (Dey et al, 2019) and 44.68% by (Dinani et al, 2019) . These values suggest that DDGS has the potential to replace soybean meal in adult dog diets. However, the variation in DDGS composition can be attributed to factors such as the grain source and drying method, which can affect both nutrient concentration and bioavailability. The experimental diets in this study, containing graded levels of DDGS (0, 5, 10, 15, and 20%), were formulated to be isocaloric and isonitrogenous, meeting the nutritional requirements for adult dogs as per ICAR, 2013.

In Vitro Digestibility of Experimental Diets

The in vitro digestibility study showed that the digestibility of dry matter (DM), organic matter (OM), CP, and EE in diets containing 5%, 10%, and 15% DDGS was comparable ($p > 0.05$) to the soybean meal-based control diet (0% DDGS). However, a significant linear and quadratic trend ($p < 0.05$) was observed with increasing DDGS inclusion levels. Based on these findings, the 10% and 15% DDGS levels were selected for the in vivo trial to further explore their impact on nutrient utilization, gut health, and immune response in adult Beagle dogs.

Body Weight Changes and Daily Dry Matter Intake

No adverse effects on body weight were observed when soybean meal was replaced with DDGS up to 15% over the 90-day experimental period. Similarly, Dinani et al., (2019) reported no significant effects on body weight or feed intake in broilers fed DDGS at 0, 7.5, 10.0, 12.5, and 15% levels. In contrast, Kumar et al., (2023) observed significantly higher body weight and feed intake in Kuroiler chickens fed 15% DDGS. The lack of

significant body weight changes in the present study may be attributed to the fact that the Beagle dogs were adults, not in an active growth phase. **Digestibility of Nutrients** The digestibility of DM, OM, CP, and EE was significantly higher ($p < 0.05$) in dogs fed 0% and 10% DDGS diets compared to those fed the 15% DDGS diet. Additionally, significant linear and quadratic trends ($p < 0.05$) were observed for these nutrients as DDGS levels increased. This is consistent with (Risolia et al, 2019), who reported that DDGS inclusion (0, 16%, and 18%) linearly reduced the digestibility of EE, gross energy, and ME in dog diets. (Khose et al., 2018) also found that DDGS inclusion up to 20% reduced ($p < 0.05$) the digestibility of DM, OM, EE, and gross energy in dogs. Similarly, Dinani et al., (2019) observed that rice DDGS at 15% in broiler diets significantly ($p < 0.01$) reduced dry matter metabolizability, gross energy utilization, nitrogen retention, and protein and energy efficiency ratios. In contrast, Reddy et al., (2020) reported no significant effects ($p > 0.05$) on nutrient digestibility in finishing pigs fed up to 40% rice DDGS. Gupta et al., (2020) also reported no adverse effects on DM metabolizability, nitrogen, calcium, or phosphorus retention in layer hens fed rice DDGS with or without enzyme supplementation. Shirisha et al., (2021) found that rice DDGS up to 14% had no effect on DM and CP retention in broiler chickens.

Blood Biochemical Parameters

All blood biochemical parameters remained comparable ($p > 0.05$) across dietary treatments at both 0 and 90 days, with no significant linear or quadratic trends ($p > 0.05$). All values were within normal physiological ranges, confirming the health of the dogs throughout the study. Similarly, Dinanai et al., (2018) reported that 15% rice DDGS in broiler diets had no adverse effects on blood parameters. Kaninde et al., (2023) also observed no significant changes in Hb, PCV, glucose, protein, albumin, cholesterol, or triglyceride levels with DDGS inclusion up to 15% in broilers. Shirisha et al, (2021) similarly found no effects on serum biochemistry in broilers fed up to 14% rice DDGS. However, contrary to these findings, Keerthana et al., (2022) observed that rice DDGS inclusion in Japanese quail diets significantly ($p < 0.05$) reduced total cholesterol levels and increased total serum protein levels.

Fecal Score

Fecal scores were statistically similar ($p > 0.05$) across all treatment groups, with no clear linear or quadratic trends. This agrees with Risolia et al., (2019), who reported no differences in fecal scores among dogs fed 0% and 20% DDGS diets with or without xylanase and protease enzymes. Silva et al., (2016) also reported no significant differences in fecal scores in dogs fed DDGS at 0%, 6%, 12%, and 16%.

Gut Health Parameters

Fecal pH, ammonia, and lactic acid levels were not significantly different ($p > 0.05$) among treatment groups, and no linear or quadratic effects were observed. These results are consistent with Risolia et al., (2019), who found no significant changes in fecal pH, ammonia, or short-chain fatty acid levels in dogs fed 0% or 20% DDGS diets with or without enzyme supplementation. Similarly, Silva et al., (2016) observed that while fecal ammonia remained unaffected, fecal pH declined with increasing DDGS inclusion.

Cell-Mediated Immune Response

The cell-mediated immune response to intradermal PHA-P mitogen was statistically similar ($p > 0.05$) across all treatment groups, with no significant linear or quadratic effects observed. These results are in agreement with (Biradar et al., 2024), who reported that immune response to Newcastle disease virus and infectious bursal disease virus remained unaffected by dietary DDGS in broilers. (Dinani et al., 2018) also reported no significant differences in PHA-P response in broilers fed 7.5%, 10.0%, 12.5%, and 15% DDGS diets.

Conclusion

DDGS can be used as a sustainable alternative protein source in adult dog diets and can safely replace soybean meal up to 10% inclusion without compromising nutrient digestibility, growth performance, health status, gut health, or immune function.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical standards

Experiments comply with the current laws of the country in which they were performed

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