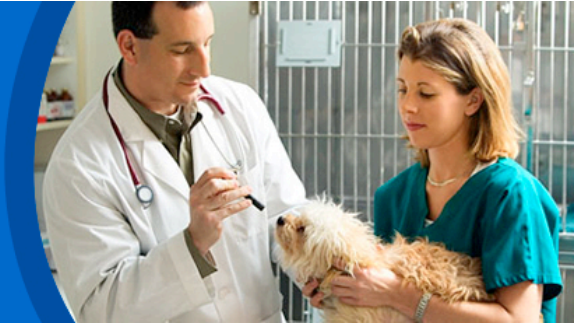




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Slaughterhouse bio-waste in canine pet biscuits: nutrition, palatability and consumer acceptance

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ABSTRACT

Slaughterhouse biowaste, often underutilized or improperly disposed of, poses environmental and health hazards. Considering this, a study was conducted to develop canine biscuits formulated by replacing 10% vegetable oil with a 10% composite of slaughterhouse waste: sheep/goat offal (1% tripe, 0.5% spleen) and poultry offal (1% intestine, 2.5% gizzard, 5% liver). While difference in moisture levels between treatment and control remained non significant, the biowaste-incorporated biscuits showed significant increase in crude protein ($11.20 \pm 0.35\%$), ash ($2.60 \pm 0.12\%$), crude fiber ($2.83 \pm 0.13\%$) and total dietary fiber ($3.29 \pm 0.33\%$). Using a 9-point Hedonic scale, although human panelists rated the treatment lower in appearance and color (7.02 vs. 8.29 , $P < 0.01$), superior texture (8.04 , $P < 0.05$) and crispiness (8.02 , $P < 0.05$) led to higher overall acceptability (8.07 vs. 7.88 , $P < 0.01$). In the palatability trials conducted, the dogs strongly preferred the treatment biscuits across all metrics, achieving a higher intake ratio (0.58 treatment vs. 0.38 control, $P < 0.01$). Thus, incorporating 10% slaughterhouse waste successfully yields a nutrient-dense, highly palatable, and acceptable canine treat.

Keywords: Slaughterhouse waste, Proximate analysis, Consumer acceptance, Canine palatability, Intake ratio

1. Introduction

Sustainable management of slaughterhouse waste is one of the major concern of the Indian meat industry pertaining to its role in environmental pollution. Bio waste from sheep and goat (tripe and spleen) along with poultry intestine, gizzard and liver, are generated in large volumes but are rarely put to economical use. Improper disposal of these bio wastes lead to Soil contamination, water pollution, greenhouse gas emissions and rising waste-handling costs for livestock facilities.

Recycling of poultry slaughter waste as a valuable source of animal protein, minerals and vitamins for feeding of dogs is highly desirable, which not only satisfies the natural instinct of carnivores, but also provides all essential amino acids, that are lacking in plant based protein (Clapper *et al.*, 2001) [1]. Liver is richer source of protein, vitamins and minerals than fresh meat. It is an excellent source of readily digestible haeme iron, vitamins particularly riboflavin, niacin, vitamin B12 and vitamin A, D, E and K. Organ meats in particular are dense in essential amino acids and micronutrients like iron, vitamin A, B-complex vitamins and carry flavour compounds that dogs (*Canis lupus familiaris*) find highly attractive. Also, Gizzard contains approximately 20% protein and could be of potential use in preparation of dog biscuits (Virk *et al.*, 2019) [2]. Efficient utilization by redirecting these offals into functional pet biscuits is a fairly natural fit for a circular-economy approach: it cuts the meat industry's waste footprint and gives manufacturers a cost-effective raw material, without necessarily compromising nutritional quality. According to Health for Animals Organization (2022), it is estimated that over half of the global population now owns a pet. In addition, the pet food market has experienced growth due to the trend of pet humanization (Verdon, 2022) [3]. This trend reflects pet caregivers' increasing demand for healthier options for their pets (Bulochava and Evans, 2021) [4].

Significant expansion of the pet food market has encouraged industry professionals to innovate their pet food products, providing pet caregivers with a broader array of choices in the market (Watson *et al.*, 2023) [5].

Although taste and olfactory perceptions are not completely similar in dogs and humans, human sensory data can be useful to assist with pet food formulation as the pet owners make decision on the purchase of the pet food products. The comparison between flavor profiles created by human panellist and data obtained from

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the pet acceptance and preference indication of the effects of ingredients and processing changes on the products (Pickering 2008). Consequently, palatability becomes a key factor driving purchasing decisions in the pet food market (Aldrich *et al.*, 2015; Tobie *et al.*, 2015; Watson *et al.*, 2023) [6, 7, 5].

With all of that in mind, developing a bio-waste incorporated nutritive, pet biscuit was perceived with these three objectives:

1. Nutritional integrity (proximate profile): crude protein, fat, fibre, ash and moisture content, following dietary guidelines for companion animals.
2. Consumer acceptance: how pet owners judge safety, hygiene, appearance and how they'd actually prefer buying a product made from slaughterhouse by-products.
3. Canine palatability: how dogs respond in terms of taste, aroma and preference, since none of the above would matter if the dogs won't eat it.

2. Materials and methods

The present study was undertaken for the formulation, proximate profile analysis, human sensory acceptability and canine palatability trials of the developed canine pet biscuit incorporated with ruminant and poultry slaughterhouse by-products.

2.1 Raw materials

- Slaughterhouse bio-waste: sheep and goat bio-waste (tripe and spleen); chicken bio-waste (liver, gizzard and intestine).
- Dough matrix and additives: Refined wheat flour (*Maida*), vegetable oil, egg, salt, sugar, baking powder and spice mix.

2.2 Preparation of slaughterhouse bio-waste powder

Cleaned sheep and goat tripe and spleen were cut, pressure-cooked for 30 minutes, and minced. The minced tripe and spleen were hot-air dried at 70 °C for 5 and 6 hours, respectively, before being pulverized and sieved into fine powders as described by Athira *et al.* (2017) [8]. Chicken liver, gizzard and intestine were cleaned separately. Intestine was washed in warm water and autoclaved (121 °C for 15 minutes), while liver and gizzard were frozen (-23 ± 1 °C) and minced. All components were then dried at 70 °C for 16–18 hours, pulverized and sieved into a fine powder, following the procedure described by Kumar *et al.* (2022) [9].

2.3 Preparation of spice mix

Commercial spice ingredients were sourced locally and cleared of foreign debris. Residual moisture was removed by gentle drying over a low flame, after which the dehydrated material was milled and passed through a fine-mesh screen. The individual powders were combined according to the ratios in Table 1, and the finished blend was stored in a PET (polyethylene terephthalate) container until use.

Table 1. Composition of Spice Mix Blend

S. No.	Ingredients	Botanical Name	Percentage (w/w)
1	Coriander	<i>Coriandrum sativum</i>	20.0
2	Aniseed	<i>Pimpinella anisum</i>	15.0
3	Black pepper	<i>Piper nigrum</i>	15.0
4	<i>Capsicum</i>	<i>Capsicum frutescens</i>	15.0
5	Cumin seeds	<i>Cuminum cyminum</i>	10.0
6	Dried ginger	<i>Zingiber officinale</i>	10.0
7	Cinnamon	<i>Cinnamomum verum</i>	5.0
8	Cloves	<i>Syzygium aromaticum</i>	5.0
9	Turmeric	<i>Curcuma longa</i>	5.0
10	Cardamom	<i>Elettaria cardamomum</i>	2.0

2.4 Formulation and preparation of canine pet biscuit

The formulation of the control and treatment biscuits is given in Table 2.

Table 2. Formulation Base for Canine Pet Biscuit

S. No.	Ingredients	Control (%)	Treatment (%)
1	Refined flour (<i>Maida</i>)	55.0	55.0
2	Vegetable oil	20.0	10.0
3	Slaughter house biowaste	0.0	10.0*
4	Salt	2.0	2.0
5	Sugar	3.0	3.0
6	Spice mix	3.0	3.0
7	Egg	16.0	16.0
8	Baking powder	1.0	1.0
Total Base		100.0	100.0

* In the formulation of the treatment biscuit, vegetable oil was partially replaced with slaughterhouse bio-waste (tripe, spleen, intestine, liver and gizzard) at a total inclusion of 10% {(sheep and goat tripe (1.0%) and spleen (0.5%), chicken intestine (1.0%), gizzard (2.5%) and liver (5.0%)}.

2.5 Proximate composition

The proximate profile analysis was evaluated on the basis of moisture content, protein content, ash content and fat content. Moisture, protein, lipid, ash and dietary fibre for both control and treatment samples were evaluated following AOAC (1995) [10] methodology. Total fat was quantified with a SOCS Plus assembly (Model SCS 4, Pelican Equipment Pvt. Ltd.), and total nitrogen/protein was estimated using a KEL Plus system (Model Classic DX, Pelican Equipment Pvt. Ltd.). Total dietary fibre was quantified using a modified AOAC (2012) [11] protocol.

2.6 Consumer acceptance

A panel of seven trained members scored the biscuits on general appearance, colour, meat-flavour intensity and overall acceptability using a 9-point Hedonic/descriptive scale, where 9 represented extremely acceptable/excellent and 1 represented poor.

2.7 Palatability and acceptability assessment

The palatability trial used six dogs of similar size and breed, run across three experiments with six replications each. Biscuits were offered 3–5 hours after normal feeding, and each dog's approach, interest in eating and eating behaviour were scored on a modified score-card (Pame *et al.*, 2017) [12], alongside the owners' own impressions of colour, odour and appearance recorded on the same sheet. Data were tabulated as mean scores across treatments.

Preference test (Two-bowl test)

A two-pan test compared the formulations directly. Six dogs were fed 3–5 hours after a meal, across six replications for each of Experiments 1, 2 and 3. Each dog received 60 g of biscuit split between two pans (A and B) with water available ad libitum. After 30 minutes, the leftover biscuit in each pan was weighed, and pan positions were swapped every 10 minutes to rule out a location bias.

Preference was calculated as an Intake Ratio, $IR = A/(A + B)$, where A and B are the amounts (in grams) consumed from each pan. An IR above 0.50 means a preference for food A, below 0.50 means a preference for food B, and 0.50 is neutral. The values reported are the average IR across the dog group.

2.8 Statistical analysis

The data generated in this study was pooled and analyzed by statistical method of Independent Sample *t*-test and Mean $\pm$ S.E using SPSS Statistics 20.0 software package developed as per the procedure of Snedecor *et al.* (1995) [13] and means were compared by using Duncan's multiple range test.

3. Results and discussion

3.1 Proximate composition

Table 3 gives the mean ($\pm$ SE) proximate composition for both biscuits. Under IS 11968:2019 [14] (Nutritional Standards for Dry Pet Food), the requirements are: moisture max 10%, crude protein min 18% for adult dog food, crude fat min 5%, crude fibre max 4.5%, and total ash max 10%. The proximate composition of the developed canine pet biscuit was within these prescribed standards.

There was an increase in the moisture content of the treatment biscuits in comparison to the control biscuits, though, the difference in the moisture content of both control and treatment biscuits were not significant. Similar observations were obtained in a study on utilization

of poultry slaughter by products to develop cost effective dried pet food (Kumar *et al.*, 2022) [9]. There was a highly significant ($P < 0.01$) increase in the total ash, crude fibre and total dietary fibre content of the treatment biscuits compared to the control biscuits. A significant increase ($P < 0.01$) was noticed in the protein, fat and energy values of the treatment than the control biscuits.

The increase in ash content, from 1.03% to 2.60%, indicates a notable rise in essential mineral and inorganic matter. This finding contrasts with Poppi *et al.* (2023) [15], who reported significant differences in moisture, lipid, ash, and caloric value in their study on the nutritional evaluation and palatability of pet biscuits: moisture was significantly higher (21.21%) and ash content lower (4.93%) in biscuits made with chicken viscera flour than in biscuits made with Nile tilapia flour or fish viscera flour.

On the other hand, the protein, crude fibre and dietary fibre increases lined up well with Hall *et al.* (2018) [16], who found that higher dietary crude protein consistently improved palatability and consumption across dogs of different sizes. Crude fibre showed a highly significant increase ($P < 0.01$) from 1.84 ± 0.17 to $2.83 \pm 0.13\%$, while total dietary fibre (TDF) numerically increased from $2.31 \pm 0.13\%$ to $3.29 \pm 0.33\%$ ($P < 0.01$). The inclusion of connective tissue matrix components (collagen and elastin from tripe and gizzard muscle walls) alongside insoluble structural elements from cleaned poultry intestinal tissues contributes to this fiber increase.

Dietary fiber plays an important functional role in canine gastrointestinal health by: Promoting normal peristalsis and stool bulk, serving as a substrate for gut microbiota fermentation (producing short-chain fatty acids like butyrate) and modulating postprandial glucose absorption. Knight *et al.* (2021) [17] reported a similar link between protein quality and digestive response, and both Meineri *et al.* (2021) [18] and Samant *et al.* (2021) [19] point to fat and protein concentration as the main levers behind how dogs respond to a food, organoleptically speaking.

Table 3. Proximate Composition of Canine Pet Biscuit Incorporated with Slaughterhouse Bio-Waste (Mean $\pm$ SE)

Parameters	Control (Without Bio-Waste)	Treatment (With 10% Bio-Waste)	'F' Value
Moisture (%)	3.95 ± 0.23	4.08 ± 0.20	0.01 NS
Protein (%)	10.23 ± 0.17	11.20 ± 0.35	3.29 *
Fat (%)	9.23 ± 0.36	7.95 ± 0.36	0.08 *
Ash (%)	1.03 ± 0.05	2.60 ± 0.12	2.93 **
Crude Fibre (%)	1.84 ± 0.17	2.83 ± 0.13	0.09 **
Total Dietary Fibre (%)	2.31 ± 0.13	3.29 ± 0.33	3.80 **
Energy (kcal/100g)	409.49 ± 2.54	401.68 ± 1.70	0.46 *

Note: $n = 6$. Means bearing different superscripts within a row differ significantly ($P < 0.01$). * $P < 0.05$ (significant); ** $P < 0.01$ (highly significant); NS = non-significant.

3.2 Consumer acceptance

Table 4 shows the Mean $\pm$ SE scores of Consumer acceptance. Incorporation of 10% slaughterhouse bio-waste significantly darkened the visual appearance of the product, resulting in lower appearance scores (7.02 ± 0.11 vs. 8.29 ± 0.08 , $P < 0.01$) from evaluators, a pattern that was well documented for baked pet treats containing organ tissue and meat by-products (Donfrancesco *et al.*, 2017; Virk *et al.*, 2019) [20, 21]. At the same time, the structural matrix (8.04 ± 0.16 , $P < 0.05$) contributed by the bio-waste components enhanced physical binding, shape retention, and surface characteristics; this textural en-

hancement aligns with the higher mineral-ash and fibre levels, which contributed to a firmer, crisper bite.

While the visual and olfactory scores of bio-waste samples were reduced relative to the colour and flavour scores of the control, the gains in crunchiness, shape and texture offset those declines, yielding a net positive shift in overall acceptability of the treatment biscuits (8.07 ± 0.12 vs. 7.88 ± 0.06 , $P < 0.01$), which fitted well with Pame *et al.* (2017) [12], who found that owner-preference attributes (odour, colour and appearance) and canine palatability attributes of shelf-stable pet kibble incorporating slaughterhouse by-products were clearly distinguishable from those of control kibble.

Table 4. Consumer Acceptance Scores of Canine Pet Biscuits (Mean $\pm$ SE)

Parameters	Control (Without Bio-Waste)	Treatment (With 10% Bio-Waste)	F Value
Appearance / Colour	8.29 $\pm$ 0.08	7.02 $\pm$ 0.11	0.01 **
Aroma / Flavour	8.07 $\pm$ 0.16	7.26 $\pm$ 0.19	4.36 *
Surface Texture and Shape	7.54 $\pm$ 0.15	8.04 $\pm$ 0.16	0.05 *
Crispiness / Crunchiness	7.57 $\pm$ 0.13	8.02 $\pm$ 0.14	1.44 *
Overall Acceptability	7.88 $\pm$ 0.06	8.07 $\pm$ 0.12	0.00 **

Note: $n = 6$. Means bearing different superscripts within a row differ significantly ($P < 0.01$). * $P < 0.05$ (significant); ** $P < 0.01$ (highly significant).

3.3 Canine palatability trials

3.3.1 Acceptability scores

Table 5 summarizes how the dogs themselves responded. The scores obtained for interest to eat (7.97 ± 0.02 , $P < 0.01$), nature of eating (7.61 ± 0.08 , $P < 0.01$) and overall preference (7.91 ± 0.04 , $P < 0.01$) were all highly significant for the treatment biscuits, except that of the approach score (7.83 ± 0.06 , $P < 0.05$), which was significantly higher. The observation in this study showing increase in the interest

to eat scores of the treatment biscuits were similar to the results obtained by Muthulakshmi *et al.* (2024) [21]. The most likely explanation is straightforward, the offal components bring volatile aromatic compounds and soluble amino acids that cereal alone doesn't offer, and dogs pick up on that quickly. Overall, the dogs showed a clear, strong preference for the bio-waste-supplemented formulation over the control, consistent with the protein and fat-driven palatability effects also noted by Hall *et al.*, 2018 [16]; Knight and Satchell, 2021 [17]; Meineri *et al.*, 2021 [18]; and Samant *et al.*, 2021 [19].

Table 5. Acceptability Scores in Canine Palatability Trials (Mean $\pm$ SE)

Parameters	Control (Without Bio-Waste)	Treatment (With 10% Bio-Waste)	F Value
Approach to Biscuit	7.61 $\pm$ 0.08	7.83 $\pm$ 0.06	18.58 *
Interest to Eat	7.52 $\pm$ 0.08	7.97 $\pm$ 0.02	278.12 **
Nature of Eating	7.16 $\pm$ 0.06	7.61 $\pm$ 0.08	18.58 **
Preference Score	7.33 $\pm$ 0.07	7.91 $\pm$ 0.04	38.30 **

Note: $n = 6$. Means bearing different superscripts within a row differ significantly ($P < 0.01$). * $P < 0.05$ (significant); ** $P < 0.01$ (highly significant).

3.3.2 Two-bowl intake ratio (IR)

Table 6. lays out the direct preference comparison. The control biscuit scored least preference below the neutral line ($IR = 0.38 \pm 0.01$), while the bio-waste treatment biscuits scored highly significantly (0.58 ± 0.02 , $P < 0.01$), comfortably above 0.50, which confirms a clear, consistent preference for the bio-waste formulation over the control.

This result tracks with Pame *et al.* (2017) [12], who reported higher IR values for pet kibble made with 20% meat-and-bone meal and 5% rendered fat, and with Dust *et al.* (2005) [22], who found that animal-derived protein and fat sources improve both the physical bite and the organoleptic appeal of companion-animal diets, translating into more positive feeding behaviour overall.

Table 6. Two-Bowl Test Intake Ratio (IR) of Canine Pet Biscuits (Mean $\pm$ SE)

Comparison	Intake Ratio (IR)	F Value
Control (Without Bio-Waste)	0.38 $\pm$ 0.01	0.814 **
Treatment (With 10% Bio-Waste)	0.58 $\pm$ 0.02	

Note: $n = 6$. Means bearing different superscripts differ significantly ($P < 0.01$).

** $P < 0.01$ (highly significant).

Conclusion

Incorporating sheep, goat and poultry offals like spleen, intestine, gizzard and liver to develop a functional canine pet biscuit at a 10% inclusion level turns the by-products that would otherwise be low-value waste into a genuinely functional, nutrient-dense and palatable pet biscuit. The formulation clears both bars that matter commercially: owners still rate it well, largely on the strength of its crispness and texture, and dogs show a clear, measurable preference for it ($IR = 0.58$), all while staying within the recommended energy contribution for a complementary pet snack.

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