ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 4, pp: (116-122), Month: October - December 2019, Available at: www.researchpublish.com

Evaluation of Effectiveness of Various Soybean Meal Processing Techniques on Protection of Protein from Rumen Degradation

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Abstract: **Soybean meal (SBM), an industrial by-product, is a rich source of true proteins for high performing domestic ruminants. Soybean meal, however, is highly degraded in the rumen by the ruminal microorganisms leading to downgrading of protein quality. Therefore, the objective of the study was to investigate the effectiveness in protection of SBM protein from rumen microbial degradation and its availability in the lower gut using different techniques at different levels. In the study, SBM (50% CP) was processed using four techniques in randomized complete block design; roasting at 150^{o}C for 0, 15, 30, and 45 minute(s); coating with 0, 10, 20, and 30 ml calcium soaps of beef oil (CSBO); and spraying with wattle tree pods extracts (WTPE) at 0, 1, 2, and 3%. Consequently, samples of PSBM were washed in tap water and incubated in rumen of a fistulated steer for 16 hours and both insoluble and undgradable protein (RUP) levels recorded. Also PSBM was incubated in distilled water and pepsin solution for 48 hours soluble and digestible protein recorded. In addition, Digestible Rumen Undegradable Proteins (DRUP) levels, derived by multiplying both RUP at 16 hours with net digestible proteins were used a complete randomized block design was used to evaluate PSBM. Roasted SBM for 30 minutes at 150^{o}C ranked the highest (P<0.05) compared to other techniques levels.**

Keywords: **Soybean Meal, Techniques, Digestible Rumen Undegradable Proteins.**

I. INTRODUCTION

Soybean meal (SBM), an industrial by-product obtained during processing of soybean oil is a rich source of true proteins, locally available. However, SBM is highly degraded in the rumen rendering it almost unavailable for the supply of proteins for intestinal digestion and utilization. For improved utilization of SBM in ruminants, it is necessary to protect it against ruminal degradation. There are extensive benefits when well treated soybean meal is fed as a protein supplement to agricultural ruminants in term of increased milk yield and improved growth [1]. For a high performing livestock ruminant, whereby a lot protein is necessary, a less degradable protein often called “rumen undegradable (RUP) is necessary [2]. The RUP is necessary and more valuable source of Nitrogen for a body with a physiological high demand for proteins than RDP [3]. The RUP plus the microbial protein produced during rumen fermentation form the sources of amino acids for a high performing ruminant livestock [4]. Several techniques are available for protecting SBM against ruminal degradation both in chemical and physical forms. During the process, there are chances of overprotected SBM and become unavailable for the supply of protein for intestinal digestion and absorption. The choice of technique of protecting SBM depend on balancing between extend of protection against ruminal degradation and availability of protein for intestinal digestion. Therefore, the objective of the study was to determine an efficient technique and level of processing SBM to minimize its degradation inside the rumen and be available at the intestinal level.

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II. MATERIALS AND METHODS

The experiment was conducted in Biological laboratories, Chuka University and Animal Nutrition Laboratory, University of Nairobi. Soybean meal was treated using four techniques in randomized completely block design (RCBD) as follows; 1) Four (4) batches of 20kg of soybean meal (SBM) (U1, U2, U3, and U4) were left unprocessed. 2) Four batches of 20 kg of SBM (R1, R2, R3 and R4,) were rosted for the following durations of time (minutes); R1 (unprocessed) for 0, R2 for 15, R3 for 30 and R4 for 45. 3) Four batches of 20 kg of SBM (C1, C2, C3 and C4) were coated with calcium soap of beef oil (weight/volume (w/v)) as follows; C1 (unprocessed) mixed with 0%, C2 mixed with 400g (2%), C3 mixed with 800g (4%) and C4 with 1200g (6%). 4) Four batches of 20 kg of SBM (S1, S2, S3 and S4) were sprayed with different amounts of wattle tree pod extract (WTPE) to provide different tannin concentration of (weight/volume (v/w)) as follow; S1 (unprocessed) (0%), S2 for 200g- (1%) S2 for 400g- (2%) and S3 for 600g- (3%).

In the same design, evaluation of the processed soybean meals (PSBMs) was conducted by first analyzing the total crude protein content (CP) of the samples. Separately, the samples were washed in the tap water and incubated in the rumen of a fisulated steer for 16 hours. The sample residues were later analysed for final CP as STW and RUP% that soluble protein in tap water and rumen undegradable protein respectively.

The PSBMs samples were also subjected to the second evaluation where, like the other method, they were first analysed for total CP before their incubation in distilled water and pepsin solution for 48 hours. After this, the samples were analysed for the final CP as, DDW and DPS% that is protein soluble in distilled water and in pepsin solution respectively. The final evaluation was done using estimated digestible rumen undegradable protein (DRUP) percentage levels. The DRUP percentages using a compleletly randomised design (CRD) were estimated by multiplying RUP at 16 hours with net digestible protein in pepsin solution

DRUP=RUP*NDPS [5].

Net DPS was the digestible protein in pepsin solution (DPS%) less soluble protein in tap water (STW%). Data was analysed according to general liner model (GLM) of each design in [6]

Data was analysed according to ggeneral liner model (GLM) of each design in [6] The computed means were subjected to analyses of variance (ANOVA) to test for any significant differences (P=0.05) on response variables. Means were separated using Fisher's least significance difference (lsd) procedure [7].

III. RESULTS

Protein Soluble in Tap Water and Undegradable in all Levels of Processing Techniques of SBM

Solubility of protein in tap water (STW) of SBM processed at all levels of processing techniques are summarized in Table 1.

TABLE 1: Protein Solubility in Tap Water (STW) (% of Initial Protein) and Rumen Undegradable Protein (RUP) of SBM Processed at Different Levels using Different Techniques

Processing technique	Levels	n	STW	+SE	RUP	+SE
Unprocessing	0	3	17.9	±0.47	27.8	±0.52
	1	3	18.1	±0.49	27.5	±0.55
	2	3	18.4	±0.52	27.0	±0.50
	3	3	18.3	±0.50	27.1	±0.47
		±SE	1.1		2.1	
Roasting (R)	0	3	18.1^{a}	±0.49	27.3^{c}	±0.49
	1	3	14.6^{b}	±1.20	36.0^{a}	±0.78
	2	3	12.3^{c}	±0.21	36.3^{a}	±0.14
	3	3	15.0^{b}	±0.21	33.3^{b}	±0.64
		±SE	1.6		1.9	
CSBO Mixing (C)	0	3	18.1^{b}	±0.49	27.3^{b}	±0.49
	1	3	20.2^{a}	±0.07	23.9^{c}	±0.99
	2	3	18.0^{b}	±0.57	29.5^{a}	±0.14

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	3	3	17.9[b]	±0.49	30.5[a]	±0.21
		±SE	2.0		1.8	
WTPE Spraying (S)	0	3	18.1[a]	±0.49	27.3[c]	±0.49
	1	3	18.7[a]	±0.21	36.3[a]	±0.71
	2	3	14.1[b]	±0.07	32.3[b]	±0.92
	3	3	14.4[b]	±0.56	30.7[b]	±1.27
		±SE	2.2		1.7	

CSBO = Calcium Soap of Beef Oil, WTPE = Wattle Tree Pod Extract, R 0,1,2,3=(Roasted SBM for 0,15.30,45 minutes),C 0,1,2,3=Mixed SBM with CSBO (%) at 0,2,4,6 and S 0,1,2,3=Sprayed SBM with WTPE (%) at 0,1,2,3

[a]Means followed by the same superscript in same column are not significantly different at (P<0.05

In unprocessed SBM, there was no significant difference in STW% due to unprocessing levels. In roasted SBM, there was differences (P<0.05) in STW% levels of due to roasting levels (Table, 1). Unroasted SBM (R0) had the highest STW and roasted SBM for 30 minutes (R3) had the lowest. In SBM mixed with calcium soap of beef oil, there was differences (P<0.05) in STW% levels of due to coating levels (Table, 1). Mixed SBM with 2% (C2) had the highest STW% while the rest were at the the same. In SBM sprayed Wattle tree pods extracts (WTPE), there was differences (P<0.05) in STW% levels due to spraying levels (Table, 1). Sprayed SBM with 1%; (S2) and unsprayed 0% (S0) had the highest STW while the rest were the same.

Rumen undegradable proteins levels (RUP) of SBM processed at varying levels of different processing techniques are summarized in Table, 1. In unprocessed SBM, there was no significant difference in RUP% due to unprocessing levels. In roasted SBM, there was differences (P<0.05) in RUP% levels due to roasting levels (Table, 1). In roasted SBM, there was differences (P<0.05) in RUP% due to roasting levels (Table, 1. Roasted SBM for 15 and 30 minutes; R1 and R2 respectively had the highest RUP and unroasted SBM 0 minutes R0 the lowest. Again in SBM mixed with calcium soap of beef oil, there was differences (P<0.05) in RUP% due to mixing levels (Table, 1). Mixed SBM with CBSO at 4% and 6%; (C2) and C3) respectively had the highest RUP and C1 the lowest. In SBM sprayed Wattle tree pods extracts (WTPE), there was differences (P<0.05) in RUP% due to processing levels (Table, 1). Sprayed SBM with 1%; (S1) had the highest RUP% and SBM sprayed with 2% (S2) the lowest.

Rumen undegradable proteins (RUP) of SBM processed at varying levels of different processing techniques are summarized in Table, 1. In roasted SBM, there was differences (P<0.05) in RUP% of due to roasting levels (Table, 1). Roasted SBM for 15 and 30 minutes; R1 and R2 respectively had the highest RUP and unroasted SBM 0 minutes R0 the lowest.Again in SBM mixed with calcium soap of bee oil, there was differences (P<0.05) in RUP% of due to coating levels (Table 1). Mixed SBM with CBSO 4% and 6%; (C2) and 9C3) respectively had the highest RUP and C1 the lowest. In SBM sprayed Wattle tree pods extracts (WTPE), there was differences (P<0.05) in RUP% of due to processing levels (Table 1). Sprayed SBM with 1%; (S1) had the highest RUP% and SBM sprayed with 2 % (S2) the lowest.

Soluble Protein in Distilled Water and Digestible in Pepsin Solution in all Levels of Processing Techniques of SBM

Results of soluble protein on incubation in distilled water (DDW) for 48 hours in all levels of processing techniques of SBM are summarized in Table, 2.

TABLE 2: Soluble Protein in Distilled Water (DDW) and Digestible Protein in Pepsin Solution of (DPS) SBM Processed using Different Techniques Levels

Processing technique	Level	n	DDW	±SE	DPS	±SE
Unprocessing (U)	0	3	5.8	1.3	75.5	2.2
	1	3	5.0	1.0	76.4	3.0
	2	3	5.6	1.6	77.7	3.5
	3	3	5.8	1.4	75.9	2.5
		±SE	1.0		4.4	
Roasting (R)	0	3	5.7[a]	1.91	76.9[b]	0.64
	1	3	2.1[d]	0.00	75.5[b]	0.28
	2	3	3.5[b]	0.28	78.8[a]	0.99
	3	3	2.6[c]	0.71	70.3[c]	0.21

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
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		±SE	2.3		3.7	
CSBO Mixing (C)	0	3	5.7[a]	1.91	76.9[b]	0.64
	1	3	4.5[a]	0.64	72.1[c]	0.85
	2	3	3.1[b]	0.07	81.8[a]	0.42
	3	3	2.5[c]	0.42	74.8[b]	1.48
		±SE	1.8		3.2	
WTPE Spraying (S)	0	3	5.7[a]	1.91	76.9[b]	0.64
	1	3	2.6[b]	0.99	70.9[d]	0.07
	2	3	2.3[b]	1.2	80.0[a]	1.7
	3	3	1.9[c]	0.92	74.5[c]	1.7
		±SE	1.1		4.8	

CSBO = Calcium Soap of Beef Oil, WTPE = Wattle Tree Pod Extract, n=no. of replicates
[a]Means followed by the same superscript in same column are not significantly different at (P<0.05

In roasted SBM, there was differences (P<0.05) in DDW levels due to roasting levels (Table, 2). Unroasted SBM (R0) had the highest DDW and roasted SBM at 15 minutes (R1) the lowest. In SBM mixed with CSBO, there was differences (P<0.05) in DDW levels due to coating levels (Table, 2). Unmixed soybean meal with CSBO at 0% (C0) had the highest DDW and SBM mixed with 6% (C3) the lowest. In SBM Sprayed with WTPE, there was differences (P<0.05) in DDW levels due to spraying levels (Table, 2). Unsprayed soybean meal with WTPE at 0% (S0) had the highest DDW and SBM sprayed with 3% (S3) the lowest.

Results of soluble protein on incubation pepsin solution (DPS) for 48 hours of processed SBM at all technique levels are summarized in Table 2. In unprocessed SBM, there was no difference due to unprocessing levels. In roasted SBM, there was differences (P<0.05) in DPS of due to roasting levels (Table, 2). Roasted SBM at 30 minutes (R2) had the highest DPS and roasted SBM for 45 minutes (R3) the lowest. In SBM mixed with CSBO, there was differences (P<0.05) in DPS levels due to coating levels (Table, 2). Soybean mixed with CSBO at 4% (C2) had the highest DPS and SBM mixed with 2% (C1) the lowest. In SBM Sprayed with WTPE, there was differences (P<0.05) in DPS due to spraying levels (Table, 2). Sprayed soybean meal with WTPE at 2% (S2) had the highest DPS and SBM sprayed with 3% (S3) the lowest.

Digestible Rumen Undegradable Proteins (DRUP)

Results of Digestible Rumen Undegradable Proteins (DRUP) are summarized in Table, 3.

TABLE 3: Rumen Undegradable Protein(RUP), Net Digestible Protein in Pepsin Solution (NDPPS) and Digestible Rumen Undegradable Protein (DRUP) of Processed SBM using Different Techniques at all Levels

Technique	Level	n	RUP %	NDPS (%)	DRUP (%)
Unprocessing (U)	0	3	27.8	57.6	16.0
	1	3	27.5	58.3	16.0
	2	3	27.0	59.3	16.0
	3	3	27.1	57.6	15.6
Roasting (R)	0	3	27.3	58.8	16.1[d]
	1	3	36.0	55.7	21.9[b]
	2	3	36.3	66.5	24.1[a]
	3	3	33.3	60.5	20.1[b]
CSBO Mixing (C)	0	3	27.3	58.8	16.1[d]
	1	3	23.9	51.9	12.4[e]
	2	3	29.5	63.8	18.8[c]
	3	3	30.5	56.9	17.4[d]
WTPE Spraying (S)	0	3	27.3	58.8	16.1[d]
	1	3	36.3	52.2	18.9[c]
	2	3	32.3	65.9	21.3[b]
	3	3	30.7	60.1	18.5[c]
				±SE	1.8

CSBO = Calcium soap of beef oil WTPE = wattle tree pod extract (WTPE), R 0,1,2,3= (Roasted SBM for 0,15.30,45 minutes),C 0,1,2,3= (Mixed SBM with CSBO (%) at 0,2,4,6)and S ,1,2,3 = (Sprayed SBM with WTPE (%) at 0,1,2,3)
[a]Means followed by the same superscript in same column are not significantly different at (P<0.05

There were significant differences (P<0.05) in DRUPdue to processing levels. Roasted SBM at 30 minutes had the highest (P<0.05) DRUP while SBM mixed with CSBO 2% (C2) and the unprocessed 0,1,2 and 3 had the lowest (Table, 5).

IV. DISCUSSION

Rumen Undegradable Protein of Soybean Meal Processed by Different Techniques at Varying Levels

[8] reported RUP of SBM meal roasted at 0 and 15 minutes as 23.2, and 26.3% (Initial CP) respectively results which are lower than in the present study of 27.3 and 36.0%. The size of particle of SBM being small and large could have caused the difference. [8] reported higher true protein for coarse-ground soybeans, compared with finely ground (32.8% vs. 31.7%) respectively.

[9] reported RUP of SBM mixed with calcium soap at a rate of 0, 10 and 25% as 76.4, 82.5 and 90.9% respectively after rumen incubation for 8 hours results, which are higher than in the present study. Use of high concentration of calcium soap and short incubation period in the rumen, could cause the variation in the earlier study. On increasing the incubation period to 24 hours RUP reduced to 38.9, 55.3 and 49.2% for the same parameters in the earlier study.

[10] reported RUP of SBM sprayed with 1.25 and 2.5% of tannin extracted from *Cistus ladaniferin* as 56 and 52% results which are lower than present study, when sprayed SBM was sprayed with WTPE at 1 and 3% respectively of 36.3 and 30.7% (Initial CP) equivalent to 72.6 and 61.4%. Use of *Cistus ladaniferin* extract could be the cause of variation. [11] reported RUP in SBM meal sprayed with 0, 1 and 5% of quebracho tannin as 40.8, 45.8 and 46% respectively, results which are higher than in the present of 27.3, 36.3 and 32.3% respectively 3.0% being compared with 5% in the earlier studies. Use of queracho powder in the earlier study could cause the variation.

[12] observed RUP coefficient in crude protein rose linearly with increasing levels of calliandra leaves unlike in the present study. [13]reported RUP of wet SBM sprayed with a mixture of both condensed and hydrolysable tannins at 1, 2.5 and 5% as 65.3, 65.4 and 67% respectively results which are close to the current study of 72.6, 64.3 and 60.7%. [13]again reported RUP of dry SBM sprayed with a same mixture of both condensed and hydrolysable at 1, 2.5 and 5% as 74.3, 71.0 and 70.0% respectively results which are higher than in the current study of 72.6, 64.3 and 60.7% in the current study. Use of dry SBM could be the cause of variation. [4] reported gas production of SBM treated with 1, 2 and 3% of quebracho tannin after incubation in buffered rumen fluid for 12 hours as 18.2, 16.2 and 16.7m/L respectively results which are higher than in the present which would be equivalent to 8.13, 8.59 and 8.56 m/L. Use of gas production could be the cause of variation could be the cause of variation. Gas production is more efficient than the *in-sacco* method in evaluating the effects of tannins. In the *in-sacco* method the tannins are diluted in the rumen after getting released from the nylon bag [4].

Protein Soluble in Distilled Water of Soybean Meal Processed by Different Techniques at Varying Levels

[9] reported protein solubility of SBM mixed with calcium soap of long-chain triglycerides as 4.4 and 3.3 % on incubation for 8 hours in a borate phosphate buffer for un mixed and 10% respectively. On incubating for 24 hours protein solubility was reported as 16.8 and 13.4% in the same solution respectively. Use of borate phosphate buffer in the earlier studies could have caused the difference.

[12] reported soluble of unprocessed SBM as 4.1% which close to the present study of 4.2%. [13]reported soluble protein in distilled water of unroasted SBM as 3.3% which is close to results in the current study of 4.2%. In the same study, soluble protein of roasted SBM for 30 minutes was reported as 3.6% which is close to results observed in present of 2.1%.

Protein Soluble in Pepsin Solution of Soybean Meal Processed by Different Techniques at Varying Levels

[14]reported digestibility of undegraded protein for SBM roasted at 130^{o}C at 30 and 45 minutes as 96.3 and 90.5% respectively which is higher than in the current study of 75.5 and 78.8%. Use of rumen residue samples in earlier study could have brought the variation. In addition, [15]reported post ruminal protein availability *in vitro* digestibility of roasted soybean for 30 minutes as 51% which is lower than results in the current study of 78.8%. [16]reported protein digestibility in ground soybeans at 110^{o}C for 20 and 40 minutes as 60 and, 72 respectively which is lower than results in the present study of 75.5 and 78.8% in 15 and 30 minutes respectively. Structural differences of the bean caused the variations.

[10] reported intestinal digestibility of SBM sprayed with 1.25 and 2.5% as 56 and 52 % results which lower than present study when sprayed at 1 and 3% as 70.9 and 80.0% use of *Cistus ladaniferin* could have caused the difference. [17]reported highly soluble fraction of unprocessed SBM and that with 10% calcium soap as 18.8 and 18.2% respectively results which are close to the preset study of 18.1 and 18.2%. In SBM sprayed with WPTE solubility reduced to 16.2% the cause could be increased strength of meal due to formation of tannin-protein complexes, pod extract providing the tannin. Low solubility reported in roasted SBM of 14.2% could have been caused by the stable compounds formed as a result of maillard reactions on exposing it to heat.

Digestible Rumen Undegradable protein

This suggests that roasted SBM at 30 minutes is the best option for processing SBM. In a study by [13]similar evaluation method was used in assessing protection level of protein form different ingredients including SBM against ruminal degradation. In the study, evaluation of both dry and wet SBM treated with various levels of different types of tannins was reported as 63.7% results which are higher than in the current study in sprayed SBM with WTPE. Use of ruminal residue samples for *in vitro* could have caused the variation.

V. CONCLUSION

All the four techniques at various levels except unprocessing protected SBM from degradation in the rumen.

Roasting at 30 minutes gave the highest DRUP value.

ACKNOWLEDGEMENTS

The authors acknowledge the following; Chuka University, National Research Funds, Kenya (NRF) for funding the research.

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