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Effects Of Dietary *Jatropha curcas* Meal on Percent Packed Cell Volume, Serum Glucose, Cholesterol and Triglyceride Concentration and Alpha-Amylase Activity of Weaned Fattening Pigs.

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Abstract: In a study of dietary effects of industrially "detoxified" *Jatropha curcas* meal (JCM) on Percent Packed Cell Volume (% PCV), Serum Glucose (SG), Serum Cholesterol (SC) and Serum Triglyceride (STG) concentration and Serum Alpha-Amylase Activity ("-AA) on pigs, thirty (15 boars and 15 gilts) eight-week old weaned Large White x Landrace cross breds were used. The pigs were randomly allocated to five dietary treatments (D1, D2, D3, D4 and D5) resulting in three boars and three gilts per dietary treatment. Each pig was individually penned to serve as an experimental unit with mean pig weight per treatment being 16.19 ±2.1Kg. Diets were both iso-nitrogenous and iso-calorific. The D1 group served as the control and was soybean meal (SBM) based. The JCM in treatment diets D2 to D5 substituted 6.25%, 12.5%, 18,75% and 25.0% of the crude protein contribution of the SBM in the diets respectively. All the pigs were fed twice daily on a restricted feeding regime. Water was availed *ad libitum*. Blood from the pigs was collected by venipuncture once a week for four consecutive weeks and used in the % PCV and serum metabolites determination. Both level of JCM inclusion in the diet and length of exposure to diet significantly (P<0,001) reduced % PCV, SG, SC and STG concentration and "-AA. Packed cell volume % ranged from 43.72%, the highest (P<0.001) for pigs on D1 (control) through 38.35%, 34.83%, 31.28% and 33.14% for pigs on diets D2 through to D5 respectively. The level of SG was highest (P<0.001) for sera from pigs on D1 at 7.43 mmol/L compared to 6.83 mmol/L for those on D5. The level of "-AA declined from 1745.11 U/L for pigs on D1 to 909.28U/L for those on D5. A similar trend was observed for STG (0.78 mmol/L and 0.60 mmol/L for D1 and D5 respectively) and SC concentration although SC did not show any statistically significant differences among the treatment groups. Sex had a significant effect (P<0.001) on % PCV with boars having a higher % PCV (37.63 % versus 34.89%) compared to gilts. Diet*length of exposure significantly lowered (P<0.05) % PCV only and diet*sex interaction significantly reduced "-AA level and SG concentration. Pigs on *Jatropha* based diets developed diarrhoea that was persistent. The general decline in serum metabolite levels with an increase in JCM in the diet seems to have been caused by both maldigestion and malabsorption of nutrients from the ileum. The negative presentations indicate that the JCM still contained some toxic principles indicating that processing did not completely detoxify the JCM.

Key Words: *Jatropha curcas*, serum metabolites, porcine packed cell volume

INTRODUCTION

In the Southern African sub-region the pig industry is the least developed compared to other livestock industries. Commercial pig production is on an intensive basis and competes for feed resources with Man. Feed costs in pig production accounts for 65-80 % of the production costs[15]. Soybean, the major protein source in pig rations also serves as a food ingredient in human diets. Consequently, pig production competes heavily for

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protein with man. Unfortunately soybean production is adversely affected by droughts that frequent the sub-region. Competition and drought induced shortages mean there is a dire need for a scientific search for a suitable complement and or substitute to soybean that thrives in the semi-arid climatic environment and poor soils that characterise most of the sub-region.

The physic nut, *Jatropha curcas*, (*Euphorbiceae* family) is adapted to marginal areas with poor soils and low (480 mm per annum, 28.5°C) rainfall[5,9] where growth is not in competition with annual food crops. A seed yield of 6 – 8 MT/ha has been reported[5] and is comparable to a yield of 5.5MT/ha in commercial soyabean production. *Jatropha curcas* meal (JCM) with 1-2% residual oil has a crude protein (CP) content of between 58–64%[12,13]. It thus has high potential to complement and or substitute soybean meal as a protein source in pig and monogastric diets. However, in addition to the thermo-labile lectins and trypsin inhibitors that are problem anti-nutritional factors (ANFs) in soybean, *Jatropha curcas* also contains toxic thermo-stable lipo-soluble phorbol esters (PEs). The PEs have to be removed or lowered to levels that do not elicit a toxic response for JCM to be used as an ingredient in livestock feeds[9,12]. Gross *et al*[6] reported that an ethanol extraction of moist-heat-treated *Jatropha* cake gave a meal that resulted in fish growing well, but mice grew at a slower rate than the group fed soybean meal.

The purpose of this study was to investigate the effect of substituting soyabean meal (SBM) with industrially 'detoxified' (solvent extracted, wet extruded, re-extracted and moist-heat treated) JCM as a protein source on percent packed cell volume (%PCV) and serum glucose (SG), triglycerides (STG) and cholesterol (SC) concentration and serum alpha amylase activity ("-AA) of weaned fattening pigs. Serum "-AA could be used as an indicator of pancreatitis or salivary gland pathology such as inflammation while SG concentration does indicate the gluconeogenetic capacity and or the degree of interference with ileal glucose absorption while STG and SC can be used as indicative of the degree of disruption of both the ileal lipid absorptive capacity and or homeostatic endogenous synthesis and or mobilisation of the metabolites.

Ethical approval: The Research Boards of the universities of Zimbabwe and Malawi approved this study respectively.

MATERIALS AND METHODS

***Sourcing of materials*:** Commercially produced (hexane extracted) SBM used in the diet formulation was obtained from Olivine Industries Limited Zimbabwe. *Jatropha curcas* seed was procured from Agri-Seed Services Zimbabwe.

Industrial 'detoxification' of Jatropha curcas seed: *Jatropha curcas* seed was shelled using a motorised sheller. The shelled *Jatropha curcas* seed (kernels) was 'detoxified' at Pymarc Pvt. Ltd and Speciality Animal Feed Company (SAFCO) all of Zimbabwe. The shelled kernels were put through an industrial mincer in preparation for the solvent extraction. The minced kernels were soaked in hexane (industrial grade) for 8 hours followed by 3 cycles of extraction at 30°C, each of 45 minutes duration. The hexane-extracted kernels were then dried in the shade followed by extraction with 95% ethanol at 35°C to reduce most of the highly lipo-soluble phorbol esters in the kernels[12]. The ethanol-extracted meal (still in the extraction pots) was heated with pressurised steam at 90°C for 30 minutes to distil off and recover the ethanol after which the meal was sun-dried.

The meal was then wet extruded (20% moisture, 126°C, at 2 atmospheres for 10 minutes). The extrusion aimed at reducing the high residual oil (22.16%) in the meal that harbours liposoluble PEs[13]. Reducing the oil content of the *Jatropha* meal (to between 1-2%) reduces the concentration of the PEs[12]. The extruded meal was re-extracted with hexane, at 30°C. The re-extracted meal, (still in the extraction pots) was steam-heated at 121°C for 30 minutes primarily to inactivate lectins and trypsin inhibitors[12] and secondly to distil off and recover the hexane. The resultant meal after air-drying in the sun was used to formulate the test diets.

Feed ingredients and diet formulation: The experimental diets were formulated to be of iso-nitrogenous and iso-calorific value. The dietary treatments met the recommendations of the National Research Council (NCR)[16] nutrient requirements for growing-fattening pigs. Ingredient and composition by chemical analysis of the dietary treatments are shown in Table 1.

Experimental animals and experimental design: Thirty (15 boars and 15 gilts) 8-week old fattening crosses of the Large White and Landrace breeds were used. The initial weight, sex of each piglet, age and parity of the dam were recorded. Using a random number table, the pigs were randomly allocated to cleaned and sanitised pens to which the five dietary treatments were randomly allocated,

Table 1: Ingredient and Nutrient Composition of the Experimental Diets Fed to Weaned-fattening Pigs During the Feeding Trial

Composition as fed basis (%)	Dietary Treatment				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize meal	73.2	73.4	73.6	73.8	73.9
SBM	23.0	21.5	20.1	18.7	17.3
JCM	0.0	1.3	2.5	3.7	5.0
	(0.00)	(6.25)	(12.5)	(18.75)	(25.0)
MCP	1.25	1.25	1.25	1.25	1.25
Limestone flour	1.40	1.40	1.40	1.40	1.40
Vit/Min Premix	1.00	1.00	1.00	1.00	1.00
L-lysine	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100
Composition By Chemical Analysis					
DM (%)	90.33	90.00	90.00	90.00	89.67
CP (%)[a]	16.31	16.41	16.63	16.33	17.25
EE (%)	4.61	4.44	4.81	5.00	5.39
Ash (%)	4.75	5.76	5.02	5.09	5.32
Energy (MJ/Kg)[b]	16.35	16.29	16.02	16.18	16.29
PEs (g/Kg)	0.00	1.04^{-5}	2.00^{-5}	2.96^{-5}	4.00^{-5}

SBM – Soybean meal
JCM – Industrially processed *Jatropha curcas* meal
MCP – Monocalcium phosphate
PE – Phorbol Esters
Figures in parentheses indicate % CP of SBM replaced with CP from JCM
[a,b]Diets were iso-nitrogenous and of iso-calorific value respectively

giving a total of 3 gilts and 3 boars (each individually penned and fed) per dietary treatment thus giving a randomised complete block design (RCBD), with blocking by sex. Each dietary treatment was therefore replicated 6 times with each piglet acting as an experimental unit. The mean pig weight per dietary treatment group was 16.19 ± 2.1Kg.

Animal management: Restricted feeding was practiced with pigs receiving half the feed portion at 0700 hours and the other half at 1600 hours. The pigs were started with 1Kg feed / animal / day which was increased weekly by 0.1 Kg to a maximum of 1.3Kg feed / animal / day. Water was availed ad libitum from an automatic nipple drinker. Bedding (clean hay) was provided daily after the daily cleaning of feeding troughs and pens.

Blood Collection: Blood was collected once weekly in the morning between 0600 Hrs to 0700 Hrs (before feeding) giving more than a 12-hour fast period helping guard against postprandial hyperglycaemia and hypertriglyceridemia[4]. Approximately 10ml of blood was drawn by venipuncture with 18G needles[10] and put in sodium fluoride coated tube (2 ml), EDTA coated tube (4 ml) and in silicone coated (plain) (4 ml) tube. The blood samples for serum metabolite determination were centrifuged at 3 000 rpm for 15 minutes to harvest the serum that was then stored at –20°C until the day of analyses.

***Determination of % PCV, serum glucose, alpha-amylase activity, triglyceride and cholesterol*:** PCV % was determined through micro-centrifugation. Determination for the serum metabolite levels was done on the ACE™ Clinical Chemistry System[20]. The ACE™ Serum Glucose Reagent, ACE™ Amylase Reagent, ACE™ Triglyceride Reagent were used in the determination of SG, "-AA and STG concentration respectively. The ACE™ Clinical

Chemistry System was used in the determination of SC with the enzymatic principle involved as is described by Allain *et al*[1] and Roeschlau *et al*[18].

Data Analysis: Data was analysed for the 4-week trial phase using the **Generalised Linear Models Procedure** of the Statistical Analysis System[19] and means were separated using the Least Squares Procedure. The model used in the analysis of variance was:

$Y_{ijklm} = \mu + T_i + S_j + W_k + T_i * S_j + T_i * W_k + e_{ijk}$

Y_{ijk}= Response variable of interest (% PCV, SG, STG and SC Concentration and "-AA)

μ = Overall mean common to all observations

T_i = Fixed effect of the i^{th} dietary treatment (i = 1,2,…..5)

S_j = Fixed effect of the j^{th} sex (j = 1,2)

W_k = Fixed effect of the k^{th} week of feeding (k = 1,2,3,4)

T_i*S_j = Fixed effect of the interaction between i^{th} dietary treatment and j^{th} sex

T_i*W_k = Fixed effect of the interaction between the i^{th} dietary treatment and k^{th} week of feeding

e_{ijk} = Random residual error.

RESULTS AND DISCUSSIONS

LSMeans for % PCV, SG, STG and SC concentration and "-AA by diet (treatment) are shown in Table 2. Table 3 shows Weekly LSMeans for % PCV, SG, STG and SC concentration and "-AA.

An increase in the JCM in the diet resulted in significant decrease (P<0.001) in % PCV, SG and "-AA (Table 2). A similar trend was witnessed (P<0.05) for STG. However no significant dietary effect on SC was observed (Table 2). Generally length of exposure to the JCM based diets (test diets) caused a decline in % PCV, "-AA, SG STG and SC concentration (Table 3). One-way diet*week interactions significantly (P<0.05) lowered %PCV only; while one-way diet*sex interactions significantly (P<0.05) reduced "-AA and SG level. Sex had a significant effect (P<0.001) with boars having a higher (37.63% versus 34.89%) %PCV. SG that generally declined with an increase in JCM in the diet was significantly affected (P<0.05) by diet. SG concentration was similar for pigs on D1 and D2 and for pigs on diets D4 and D5 respectively. Pigs on D3 had 7.68 mmol/L SG level, the highest, (P<0.05) and differed significantly from the rest (Table 2). Treatment differences for "-AA existed between the sera of pigs on D1 (control) and all other groups (Table 2). However, similarities in "-AA were observed between the sera of pigs on diets D2 and D3 and among the sera of pigs on diets D3, D4 and D5 (Table 2). Statistically significant differences (P<0.05) in STG concentration existed between the sera from pigs on D1 that had the highest STG concentration versus that of pigs on diets D2 through to D5 that had statistically similar (P>0.05) levels of STG. LSMeans for % PCV, "-AA, SG STG and SC by week (length of exposure) all show a decline with increase in time of exposure to the test diets (Table 3). PCV % was highest (P<0.001) at 39.87% during week 1 but was similar and lower (P>0.05) for weeks 2 and 3 (Table 3). "-AA was statistically similar between weeks 1 and 2; the same similarity was observed between weeks 3 and 4.

Interesting to note was the occurrence of diarrhoea in JCM fed pigs from day 14 of the trial. The diarrhoea was persistent up to the end of the trial. At its onset watery stools were produced but later (day 23) it became bloody. Gross inspection showed that the diarrhoeal stools had a lot of undigested ingesta. During the third week of the trial some pigs on diets D2 through D5 (with JCM inclusion) developed a skin irritation particularly around the ears. There was a 20% mortality of pigs by the end of the trial (1, 1, 2 and 2 pigs on diets D2, D3, D4 and D5 respectively). Post-mortem findings on all pigs were characteristically similar. Carcasses were pale and the stomachs were either empty or had large quantities of raw ingesta. Mild gastric fundic ulcers were noted in all cadavers. The ilea were petechiated and bloody. The colons were found to be haemorrhagic and most caeca were filled with blood clots. Profuse watery fluid was in the recta of all cadavers. However lymph nodes appeared grossly normal.

The 43.72 % PCV for pigs on D1 (control) is consistent with the average porcine %PCV of 42 % and 44 % reported by Jain[10] and Kaneko [11] respectively. However the decline in % PCV with an increase in JCM in the diet (Table 2) indicates blood loss and or destruction of erythrocytes. Paleness of the cadavers and a general decline in % PCV with an increase in exposure time to *Jatropha* based diets all point to the development of anaemia in the pigs. These results are in conformity with the preliminary findings of Chivandi *et al*[2] who reported that the haematological profile of *Jatropha* fed pigs to be characterised by early stage iron deficiency anaemia. The anaemia could have been haemorrhagic as witnessed by blood loss through the gastrointestinal tract (GIT). Furthermore damage to the GIT as indicated by persistent diarrhoea and ulceration and petechiation could also have resulted in maldigestion and malabsorption of nutrients required for erythropoiesis.

Amylase in animals comes from the pancreas and other extra-pancreatic sources such as salivary glands and duodenal mucosal cells in pigs[4]. In pigs the pancreas contributes significantly to the circulating amylase levels[17]. The decrease in "-AA with an increase in JCM

Table 2: LSMeans for Percent Packed Cell Volume (% PCV), Serum Glucose (SG) (mmol/L), "-Amylase Activity ("-AA) (U/L), Triglyceride (STG) (mmol/L) and Cholesterol (SC) (mmol/L) By Diet

Parameter	PCV	SG	"-AA	STG	SC
Diet					
D1	43.72[a]	7.43[b]	1745.11[a]	0.78[a]	2.68[a]
D2	38.33[b]	7.36[b]	1285.72[b]	0.61[b]	2.26[b]
D3	34.83[c]	7.68[a]	1145.22[bc]	0.59[b]	2.61[ab]
D4	31.28[d]	6.89[c]	972.50[c]	0.58[b]	2.62[ab]
D5	33.14[d]	6.83[c]	909.28[c]	0.60[b]	2.60[ab]
Grand Mean	36.33	7.19	1216.50	0.63	2.55
Significance Level	***	***	***	**	n.s.
C.V. (%)	8.50	8.65	29.74	32.32	21.83
R-Square	0.78	0.54	0.55	0.41	0.28
SE	0.75	0.15	88.28	0.05	0.14
Sex	***	n.s.	n.s.	n.s.	n.s.
Week	***	***	n.s.	***	**
Week * Diet	**	n.s.	n.s.	n.s.	n.s.
Sex * Diet	n.s.	**	**	n.s.	n.s.

[abcd]Within column means with different superscripts are significantly different
[***]Significant at P<0.001; [**]Significant at P<0.05; [n.s.]Non-significant

Table 3: Weekly LSMeans For PCV (%), SG (mmol/L), "-AA (U/L), STGs (mmol/L) and SC (mmol/L)

	Blood parameter				
	PCV	SG	SC	STG	"-AA
Week					
1	39.87[a]	7.89[a]	2.81[a]	0.78[a]	1556.60[a]
2	37.90[b]	7.67[b]	2.79[a]	0.77[a]	1331.30[ab]
3	36.50[b]	7.15[c]	2.47[b]	0.65[b]	1170.90[b]
4	34.39[c]	6.74[c]	2.40[b]	0.47[c]	1132.50[b]
SE	0.58	0.12	0.10	0.04	67.46

[abc]Within column means with a different superscripts are significantly different at P<0.05.

in the diets could have been due to the suppression of the exocrine pancreas. It could also have resulted from the decrease or death of duodenal mucosal cells that also secrete amylase. Studies in growing pigs and rats have shown that the production of pancreatic amylase is very sensitive to changes in dietary carbohydrates[7] and the JCM could have altered exocrine pancreatic amylase secretion. The source of the serum amylase determined in our study was not ascertained.

STG mainly come from dietary input through absorption; with some originating from adipose tissue catabolism. The decline in STG points to poor supply from the GIT. Interplay of maldigestion and malabsorption is very likely. Malabsorption may have developed secondary to maldigestion, or resulted from intestinal lesions characterised by decreased ileal absorptive surface area as a result of the death of ileal mucosal enterocytes and or interference with venous or lymphatic drainage and or distension of the lamina propria cells. Duncan *et al*[4] reported that diarrhoea and weight loss, which were conspicuous in this study, characterise the maldigestion/malabsorption syndrome. Although transient diarrhoea is generally linked with weaning[14,8]

it is not persistent and bloody as was the case in *Jatropha* fed pigs in this study.

Kaneko[11] reported that normal fasting porcine SG concentration fall within the range 4.72 to 8.33 mmol/L. The within range SG from pigs on diets D2 through to D5 maybe ascribed to homeostatic gluconeogenic response. Glucagon, growth hormone and corticosteroids as a response to pain or other stress factors are among gluconeogenic factors[4]. The diarrhoea observed in the pigs on diets D2 to D5 imposed stress during the trial resulting in a corticosteroid induced gluconeogenic glucose release from reserves resulting in a masking effect thus giving seemingly normal SG concentration across dietary treatments. A lack of definite trend in SC level on pigs on different diets seems to point out that the metabolite was influenced by other factors outside dietary effects. In addition up to 70% of the plasma cholesterol is synthesized endogenously hence the negative effect of the JCM based diet on the GIT may have had little impact on circulating levels of SC.

The skin lesions observed were similar to those described by Smith and Miles[21] characteristic of saponin induced phyto-toxicity causing photosensitization. The JCM tested negative for both saponins and lectins hence some toxic principle other than lectins and saponins was responsible for the toxicity. The residual 0.80 mg/g PEs in the JCM[2,3] compared to the 6.50 mg/g PEs in the raw *Jatropha curcas* kernels is higher than the 0.11 mg/g PEs reported by Makkar and Becker[12] in the non-toxic Mexican variety. The residual PEs are likely to have caused the toxicity or some entity, for example, curcin, curcanoleic acid (all found in *Jatropha curcas*) could have elicited the toxic response. The acute negative responses shown by the *Jatropha* fed pigs confirm its toxicity.

Conclusion: Dietary JCM caused severe adverse effects in pigs. This indicates that the detoxification procedure failed to completely remove and or neutralise the toxic anti-nutritional factors (ANFs) in the JCM. Some of the toxicity observed can be ascribed to the residual PEs in the JCM. The recovery of negatively affected pigs upon withdrawal of the JCM based diets give scope for further research into utillisation of this high potential plant in livestock feeds. It is imperative that an economical, effective and efficient detoxification method be developed.

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