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1 **True digestibility of phosphorus determined by regression method for rapeseed meal**

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13 **ABSTRACT**

14 One-hundred and forty-four male broiler chickens at 26 d old were employed to determine the
15 ileal true phosphorus digestibility of a rapeseed meal (RSM). The broiler chickens were brooded
16 together and received diets meeting nutrient recommendations from d 0 to 21. On d 21, the
17 broiler chickens were weighed and allocated to three treatments (eight replicates and six broiler
18 chickens per replicate) in a randomized complete block design. The three diets were maize starch
19 and dextrose-based semi-purified diets in which RSM was added at the rates of 450, 560, and
20 670 g/kg as the sole source of P. All the broiler chickens were euthanised on d 26 and digesta
21 from the distal ileum were collected for chemical analysis. Apparent ileal P digestibility tended
22 to increase linearly ($P = 0.074$) with increasing level of RSM in the diet. Intake of total and ileal
23 digestible P, as well as total P output, increased linearly ($P < 0.01$) with increasing dietary P
24 supplied by RSM. Regression of P output against dietary P gave the regression equation:
25 $Y = 0.575x + 1.140$ with the estimate of true P indigestibility being 57.5%. Consequently the
26 ileal true P digestibility for the RSM was calculated to be 43%. It was concluded that RSM can
27 be a substantial P source along with its use as a source of protein and energy for broiler chickens.

28 *Keywords:* Broiler chickens, Ileal, Phosphorus, Rapeseed meal, True digestibility

29

30 **1. Introduction**

31 Poultry feeds are formulated using mainly cereal grains and oilseed meals. Efficient
32 utilization of these raw materials depends on an accurate understanding of their nutritional value.
33 Phosphorus is one of the most nutritionally important minerals and expensive ingredients in
34 poultry diets. Much of P in plant feedstuffs is bound to phytate that is less available to poultry
35 (Broz and Ward, 2007; Summers et al., 1983). Therefore, inorganic P is supplemented to poultry

36 diets to meet P requirement. Because of the confusion that can result from using multiple
37 terminologies to define dietary P and its utilization, WPSA (2013) suggested using digestible P
38 for assessing dietary P and provided a protocol for estimating digestible P in feedstuffs. Few
39 studies have provided information about apparent P digestibility in rapeseed or rapeseed meal
40 (RSM) and true P digestibility (TPD) of canola meal for pigs (Akinmusire and Adeola, 2009;
41 Rodehutsord et al., 1997) but there is a dearth of such information for poultry. Therefore the
42 objective of the current study was to determine TPD in a RSM.

43 **2. Materials and Methods**

44 All the animal experiment procedures used in the current study were approved by the
45 Scotland's Rural College's Animal Experiment Committee. A total of 144 Ross 308 broiler
46 chickens were used for the experiment. The broiler chickens were brooded together in a floor pen
47 from hatch to 21 d of age during which time they received a standard commercial diet that meets
48 Ross 308 nutrient specification (<http://en.aviagen.com/ross-308/>). On d 21, broiler chickens were
49 weighed and allocated to three treatments in a randomized complete block design. Each
50 treatment had eight replicates and six broiler chickens per replicate. The ingredient and chemical
51 compositions of the experimental diets are shown in Table 1. The three semi-purified diets had
52 titanium dioxide as an indigestible marker, and contained graded levels of RSM as the only
53 source of P. The graded levels of RSM resulted in increasing level of total P in the three diets.
54 The diets were fed for 5 d, the broiler chickens were euthanized on d 26 and digesta were
55 collected from distal half of the ileum (WPSA, 2013).

56 Diets, RSM, and ileal digesta were analyzed, as appropriate, for dry matter, N, minerals,
57 Ti, phytate P, and gross energy using AOAC (2006) methods. Minerals were analyzed using
58 inductively coupled plasma – optical emission spectroscopy (Method 990.08; AOAC, 2006)

59 following digestion, in turn, in concentrated HNO₃ and HCl. Glucosinolate in RSM was analyzed
60 using ISO method 9167-1 (ISO, 1992).

61 The apparent digestibility data (calculated using the index method) were analyzed by the
62 GLM procedure of SAS 9.3 (SAS, 2011). Linear and quadratic effects of RSM inclusion levels
63 on all P utilization responses were evaluated using orthogonal polynomial tests. Phosphorus
64 intake (g/kg DM) and P output (g/kg DM) were calculated as described previously (Adebiyi and
65 Olukosi, 2015), and P_{DMO} (g/kg DM output) data were regressed against P intake (g/kg) using
66 REG procedure of SAS 9.3. Ileal TPD value was derived as described previously (Adebiyi and
67 Olukosi, 2015).

68 **3. Results**

69 The analyzed total P in the diets (Table 1) showed that the expected dietary P levels were
70 met. The RSM contained (g/kg DM) 370, 10.2, 5.8, and 8.1 of crude protein, total P, phytate P,
71 and Ca, respectively. In addition glucosinolate content for the RSM was 11.2 µm/g whereas
72 sinapine and tannin contents were 4.7 and 2.11 mg/g, respectively. The analysis showed that
73 approximately 57% of total P in the RSM is phytate P and that glucosinolate, sinapine, and
74 tannin levels were within the levels expected of the meals from modern varieties of rapeseed.

75 Table 2 shows the data for apparent digestibility of the experimental diets. Ileal DM
76 digestibility decreased linearly ($P < 0.01$) with increasing level of RSM in the diets. There was
77 linear increase ($P < 0.01$) in ileal digestible P, total P intake, digestible P intake, and P output
78 with increasing dietary level of RSM. Apparent P digestibility tended to increase linearly ($P =$
79 0.07) with increasing dietary level of RSM. There were no quadratic effects of increasing RSM
80 level in the diet on any of the measured responses.

81 The regression of dietary P (g/kg DM) against P output (g/kg DM output) produced the
82 linear equation: $Y = 0.575x + 1.140$. The slope of the regression equation (b , 0.575) is an
83 estimate of coefficient of P indigestibility and the intercept 1.14 is an estimate of the endogenous
84 P loss (g/kg DM intake). The standard error of the linear term was 0.124 and that of the intercept
85 was 0.825 with r^2 of 0.61. Coefficient of TPD was calculated as: $1 - 0.575$, and gave a value of
86 0.425 (or 43%). True digestible phosphorus for the RSM was derived as the product of total P
87 content (10.2 g/kg DM) of the RSM and its TPD coefficient (0.43) and gave a value for true
88 digestible P of 4.39 g/kg (DM).

89 4. Discussion

90 The objective of the current study was to determine the TPD of an RSM for broiler chickens
91 by means of the regression method using protocol similar to that developed by WPSA (2013),
92 with the exception that egg albumin was not used in the diets fed in the current experiment. The
93 value for TPD estimated for the RSM in the current study is 43%. The value is close to, though
94 numerically lower than, the 47% estimated for canola meal by Mutucumurana et al. (2014). In
95 pigs, Akinmusire and Adeola (2009) reported a TPD of 34% for canola meal whereas
96 Rodehutsord et al. (1997) reported apparent digestibility values of 42 or 24% for full fat
97 rapeseed or solvent extracted rapeseed meal, respectively.

98 The level of total P in the RSM investigated in the current study was 10.2 g/kg and the
99 phytate P was 5.8 g/kg thus the non-phytate P is 4.4 g/kg or 43% of total P. The true ileal
100 digestible P (calculated as product of coefficient of TPD and total P in the RSM) was 4.39 g/kg.
101 Consequently the total digestible P (4.39 g/kg) was virtually the same as total non-phytate P (4.4
102 g/kg) content in the meal. It was assumed, for the calculations of total digestible P above, that all

103 the non-phytate P in the RSM was absorbed. This is probably not true, and hence it is possible
104 that the contribution of phytate-P to the total digestible P in the sample was greater than zero
105 value suggested by the calculations above. In all probability, a small portion of the phytate-P in
106 the RSM contributed to the true digestible P (4.39 g/kg) calculated above. This suggests that very
107 little phytate-P was digested, and hence there is considerable opportunity for phytase to liberate
108 P from phytate in RSM.

109 The methodology used for calculating TPD in the current study is the approach proposed
110 by Dilger and Adeola (2006), which involves estimation of true P indigestibility from the slope
111 of the regression of P output (g/kg DM output) against dietary P level. By definition, TPD is
112 calculated as 1 minus true P indigestibility. On the other hand, TPD can be calculated directly
113 from the slope of regression of digestible P intake (g/kg DMI) against dietary P level as
114 suggested by WPSA (2013). Because the data used to generate digestible P intake can also be
115 used to calculate P output, it is possible to run the two regression analyses and compare the
116 outputs; and by definition, the slope of one method should be 1 minus the slope of the other
117 method; whereas the intercept should be the same, although with different signs.

118 A requirement for using linear regression analysis to estimate true digestibility is that
119 there must be a statistically significant regression coefficient and it is important to ensure that
120 this requirement is met before proceeding further with the use of linear regression. However, an
121 inherent issue with regression analysis is the possible presence of outlying observations or
122 influential data points. Instances of such data points can produce erroneous estimates of true
123 digestibility and EPL and thus make comparison across studies difficult. It is therefore important
124 to ensure that data are checked for such issues before proceeding with the use of linear
125 regression.

126 Regression of P output against dietary P in the current study gave an estimate of EPL as
127 1.14 g/kg DMI (standard error was 0.83), whereas regression of digestible P intake against
128 dietary P gave an EPL estimate of -1.14 g/kg DMI. The standard error of EPL estimate was
129 relatively large and this might be responsible for the relatively wide confidence interval and the
130 lack of statistical significance. It would be useful in future estimates to indicate whether these
131 estimates of EPL are different from zero especially in cases where enzymes supplementation
132 improved digestibility of the nutrient. This will help indicate how much of the improvement in
133 digestibility in response to the enzyme was due to reduction in EPL. For example, Akinmusire
134 and Adeola (2009) showed that phytase supplementation increased TPD of canola meal from 34
135 to 61% and decreased EPL estimate from 101 to 38 mg/kg DMI. At first glance, there appears to
136 be a drastic reduction in EPL due to phytase supplementation but, as the authors pointed out, the
137 estimates were not different from zero and hence it could be concluded that the improvement in
138 TPD as a result of phytase supplementation was not driven primarily by a reduction in EPL.

139 **5. Conclusion**

140 It is concluded from the current experiment that the coefficient of ileal TPD for the RSM
141 tested is 0.43. Therefore, less than half of the total P in the RSM was digested at the ileal level
142 and, consequently, there is potential for further P digestibility with phytase supplementation. In
143 view of the above, RSM can serve as a P source in addition to being a source of protein and
144 metabolisable energy for broiler chickens.

145 **Conflict of interest**

146 We confirm there was no conflict of interest.

147

148

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160

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187 **Table 1**

188 Ingredient composition (g/kg) and calculated analysis of the experimental diets (g/kg as fed).

Item	Diet rapeseed meal content		
	450 g/kg	560 g/kg	670 g/kg
Rapeseed meal	450	560	670
Maize starch	406	294	182
Dextrose	100	100	100
Soybean oil	20	20	20
Limestone	7	9	11
DL-Met	2	2	2
Vitamin- trace mineral premix ^a	10	10	10
Titanium dioxide	5	5	5
Total	1000	1000	1000
Chemical composition, calculated (g/kg, dry matter basis)			
Dry matter (analysed)	911	921	926
Crude protein	188	231	275
Ca	5.9	7.4	8.9
Total P ^b	5.0	6.2	7.4
Phytate P	2.9	3.5	4.2

189 ^a Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 IU;
190 vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic
191 acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg;
192 thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11
193 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

194 ^b Analysed total P was 5.6, 6.7, and 7.6 g/kg (DM) for diets with RSM contents of 450,
195 560, and 670 g/kg, respectively

196 **Table 2**

197 Ileal nutrient digestibility by the broiler chickens receiving the experimental diets.

Item	Diet rapeseed meal content			Pooled SEM	Contrasts	
	450 g/kg	560 g/kg	670 g/kg		Linear	Quadratic
Dry matter digestibility	63.3	61.3	57.9	2.1	0.001	0.426
Apparent P digestibility	21.6	26.3	26.9	4.0	0.074	0.362
Digestible P, g/kg DMI	1.21	1.77	2.03	0.15	0.003	0.508
Total P intake, g	17.8	22.1	24.4	0.7	0.001	0.783
Total P intake, g/kg DMI	5.96	6.73	7.56	-	-	-
Digestible P intake, g	3.85	5.76	6.41	0.40	0.004	0.370
P output, g/kg DMI	4.39	4.96	5.52	0.13	0.001	0.487

198 SEM – standard error of the mean; DMI – dry matter intake