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## FINAL REPORT

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Dr Reza Barekatain

# Sub-Project Title: Interactions of canola seed source, pellet temperature and fibre for broilers

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# Chapter 1 General introduction

The use of canola seed in poultry diets has become common following an increase in price of fat and oil for use in broiler feed due to increased demand from biofuels and human foods. Subsequently, broiler diets have increased in cost. Many Australian broiler feed producers are including full fat CS in broiler diets. Canola seed can contribute more to the metabolisable energy content of the diet than oil-extracted solvent or expeller canola meal. This is due to the 40% oil content and 21-23% protein (Fenwick and Curtis 1980) thus making it attractive in poultry feed. The level of inclusion is typically below 8% the amount for complete removal of supplemental oil as there are concerns over glucosinolate and isothiocyanate content. Little information is available regarding the variation and nutritive value of CS grown in Australia. There are reports indicating that grinding and heat treatment of CS may enhance nutrient utilization (Muztar, et al., 1978, Salmon, et al., 1988). It is hypothesised that disruption of the cell structure results in degradation of oil containing bodies within the cell which improves oil digestibility. The current subproject was proposed based on the research gaps outlined in previous CRC sub-project 2.1.8. In particular, there was a need to further examine the variation between canola seed sources and the effect of pellet temperature and dietary insoluble fibre on utilisation of nutrient of CS for broilers. In addition, the effect of fibre and pellet temperature on fat digestibility and apparent metabolizable energy of the diets were to be investigated.

The results of a previous experiment funded by the Poultry CRC (2.1.8) showed that despite a desirable outcome in bird performance and economics of broiler diets containing FFC, these diets were not fully utilised when steam pelleted and therefore feed consumption and body weight was adversely affected. In addition, pre-grinding of the seed did not improve fat utilisation or bird performance when diets were cold pelleted but there were marginal performance improvements when diets were steam pelleted. It was hypothesized that pellet temperature and differences in seed myrosinase and glucosinolate levels were likely determinants of canola seed utilisation, particularly with respect to feed intake. The degree to which pelleting temperature affected nutritive values of canola seed was not fully understood. Furthermore, canola contains the enzyme myrosinase that catalyses the breakdown of glucosinolate to other metabolites including isothiocyanate which has an undesirable effect on palatability due to bitter taste. This activity of this enzyme can be reduced by applying heat to denature it.

The recent work by Barekatain and Swick (CRC 2.1.8 final report) indicated reduced oil digestibility in canola seed diets. The authors commented that perhaps the oil in CS is trapped in oil bodies surrounded by a peptide coating that may limit digestibility of the oil. Ways of breaking the peptide coating could include milling of the whole canola seed prior to diet addition, use of exogenous protease enzymes, or addition of insoluble fibre to stimulate gizzard activity. This project proposed to investigate to use of oat hulls as a rich source of insoluble NSP, building on results of other Poultry CRC-funded projects in Norway and Australia. If fat digestibility is increased by addition of oat hulls, then this is likely to lead to benefits when other whole oil seeds are used commercially.

The beneficial impact of inclusion of insoluble fibre in the diet of broiler chickens has been well demonstrated in literature. Studies conducted with broilers have shown that moderate amounts of dietary fibre result in significant improvement in utilisation by birds of most nutrients (Amerah, et al., 2009, Mateos, et al., 2012). In addition, Gonzalez-Alvarado et al. (2007) and Kalmendal et al. (2011) showed an increase in fat digestibility when fibre was included in broiler diets. The interaction between insoluble fibre sources (oat hulls, whole wheat) with digestibility of full fat CS has not been investigated. It is assumed that fibre results in a more developed gizzard and intestine in birds. It is hypothesised that there is increased scope for improved intake through nutrient release and lower carbohydrate solubilisation in wheat based diets which contain CS. If this hypothesis is proven successful, we can conclude that CS can be used in lieu of supplemental oil in broiler diets without compromising feed intake or body weight. Furthermore, there is no need for pre-grinding which otherwise could be cumbersome and costly due to seed size and high oil content.

This project was conducted with the following aims and objectives:

- To investigate the degree to which temperature may affect oil and energy utilisation of canola seed in broilers
- Identify the variation between different sources of canola seed in Australia in terms of nutrient composition AME and the relationship between nutrient and AME
- To investigate the role of dietary fibre content on nutrient utilisation of canola seed in broilers at different pellet temperatures
- To examine the interaction between dietary insoluble fibre and canola seed in terms of performance and nutrient utilisation in particular fat and AME

# Chapter 2 Effect of seed source and pelleting temperature during steam-pelleting on apparent metabolizable energy value of full-fat canola seed for broiler chickens

**M. Toghyani, R.A. Swick and M.R. Barekatain**

**Abstract**

Eleven canola seed (CS) samples were collected from different commercial feedmills and crushing plants in Australia and analysed for nutrient profile. Six of these samples were selected to determine the effect of seed chemical composition and pellet temperature (PT) during steam-pelleting on apparent metabolisable energy corrected for nitrogen (AMEn) values of CS for broiler chickens using a 6 × 2 factorial arrangement of treatments. The CS samples were incorporated into a corn-soybean meal diet at 15% by replacing energy-yielding ingredients and diets were steam pelleted at either 75 or 90°C. A total of 420 18-day-old male broiler chicks (Ross 308) were assigned to 14 experimental diets replicated 6 times, with 5 chicks per cage. After a 5-d diet acclimation period from d 18 to 22, excreta were collected for 72 hours. The substitution method was used to determine AME which was also corrected to zero N balance to obtain AMEn. There was no interaction of seed source and PT for ileal digestible energy (IDE), AME and AMEn values of CS ($P$ > 0.05). PT did not affect energy availability of CS ($P$ > 0.05) but increasing the PT improved pellet durability index of the diets by approximately 5.0 percentage points. A significant effect of seed source was detected for all the energy utilization values of CS ($P$ < 0.05). The IDE, AME and AMEn values of seed samples ranged from 5,239 to 5,645, 4,728 to 5,071, and 4,501 to 4,791 kcal/kg of DM, respectively. The mean AMEn values were 4,664 kcal/kg of DM, indicating a 5.7 % reduction compared with AME values. There was a negative correlation between protein and fat content of the seeds (r = -0.93, $P$ = 0.001), and consequently AMEn (r = -0.32, $P$ = 0.009). AMEn values were positively correlated with fat content of CS (r = 0.64, $P$ = 0.001). These results indicate that fat and protein content and fibre components may have a considerable effect on energy availability of CS for broiler chickens.

**Keywords:** full-fat canola seed, pelleting temperature, steam pelleting, apparent metabolizable energy, broiler chickens

## INTRODUCTION

During the past decades, rapeseed production, including canola varieties, has surpassed peanut, sunflower and even cottonseed in production, and ranks second among oilseed crops worldwide (USDA, 2014). Canola seed is now the third-largest crop (after wheat and barley) produced in Australia; its production has grown from 1.9 to 3.6 million metric tons/year over the past 5 years, making the country world's second largest exporter of canola seed (Seberry et al., 2013; USDA, 2014).

Canola seed (**CS**) is an economic feed ingredient containing well-balanced protein (19 to 22%) and a high oil content up to 45% in some cases (Shen et al., 1983; Meng et al., 2004, 2006). The oil content in full-fat canola seed (**CS**) not only can constitute up a considerable amount of energy of the diet, but it can also be an excellent source of α-linolenic acid (18:3ω3; 10%), which along with its derivatives eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3), have been shown to be important for human health, being deposited into the eggs or meat products (Ajuyah et al., 1991). However, incomplete rupture of the seed structure during feed processing may reduce the feeding value of energy yielding components due to nutrient-encapsulation. Application of cell-wall degrading enzymes has been reported to improve CS nutritional value by improving feed conversion ratio, DM, NSP digestibility, and TMEn content (Meng et al., 2006). In addition, the disruption of seed structure through grinding and pelleting would result in degradation of subcellular lipid globules and consequently improved oil digestibility (Barekatain et al., 2015). Earlier reports have also shown that grinding and heat treatment of CS enhance the nutrient utilization of FCS (Muztar et al., 1978; Salmon et al., 1988) and steam-pelleting was shown to enhance the nutritive value of CS in maize and soybean meal diets (Shen et al., 1983). The nutrient composition and presence of anti-nutritional factors in canola seed may can also affect its quality and feeding value for poultry (Khajali and Slmoniski, 2012). Apart from the oil content of

CS, its concentration of dietary fibre, protein, tannins, and glucosinolate are of concern.

In a recent experiment, Barekatain et al. (2015) showed that fat digestibility of diets containing CS was more impaired in steam pelleted diets compared to cold pelleted diets. The possible impact of pellet temperature during steam pelleting and the effect of CS nutrient profile on metabolisable energy value of the seeds for broiler chickens has not been fully investigated. The objective of the current study was to evaluate the chemical and nutritive composition of full-fat canola seeds collected from different geographical locations in Australia, investigate the effect of low (75°C) and high (90°C) pellet temperature during steam pelleting on energy utilization of 6 selected CS samples differing in chemical composition.

## MATERIALS AND METHODS

### *Seed Collection*

A total of 11 samples of CS were sourced from different location in Australia. The source of seeds is shown in Table 1 along with their chemical composition. Upon receipt of the CS samples, subsamples were obtained from spatially separated sections of each of the bulk bags using a sampling probe, and a thrice-rifled representative composite sample for each CS was used for the chemical analyses (conducted in duplicate). Homogenous subsamples of CS were analysed for dry matter, crude protein, total lysine, reactive lysine, crude fat, crude fibre, tannins, acid detergent fibre (**ADF**), neutral detergent fibre (**NDF**), glucosinolates, ash, calcium, phosphorous and sulphur (Table 1) and also amino acid composition (Table 2).

### *Birds and Housing*

All experimental procedures were reviewed and approved by the Animal Ethics Committee of the University of New England, Australia. A total of 450 day-old Ross 308 male broiler chicks were obtained from a commercial hatchery. Chicks were reared and housed in battery brooders in a room with continuous fluorescent lighting.

Table 1 Analysed chemical composition of canola seed samples (% of as is, unless specified otherwise)[3]

| Composition % | Canola seed samples | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Selected seeds for AME assay | | | | | | | | | | | | | | |
| | Seed 1 | Seed 2 | Seed 3 | Seed 4 | Seed 5 | Seed 6 | Seed 7 | Seed 8 | Seed 9 | Seed 10 | Seed 11 | Max | Min | Mean | CV % |
| DM | 94.8 | 94.1 | 94.9 | 94.2 | 94.1 | 95.0 | 94.2 | 94.2 | 94.0 | 94.1 | 94.3 | 95.0 | 94.1 | 94.3 | 0.38 |
| Crude protein | 22.5 | 19.3 | 17.2 | 21.3 | 24.1 | 20.2 | 21.5 | 21.5 | 22.5 | 23.1 | 23.1 | 24.1 | 17.2 | 21.5 | 9.19 |
| Crude fat | 41.2 | 45.9 | 47.9 | 42.2 | 41.6 | 45.8 | 42.4 | 43.0 | 40.8 | 40.4 | 40.7 | 47.9 | 40.8 | 42.9 | 5.86 |
| Crude fibre | 16.5 | 15.7 | 17.3 | 16.3 | 16.9 | 18.1 | 15.6 | 17.2 | 19.3 | 17.9 | 15.2 | 19.3 | 15.2 | 16.9 | 7.24 |
| NDF[1] | 31.6 | 25.3 | 34.4 | 27.9 | 28.1 | 26.6 | 33.9 | 28.3 | 25.9 | 34.2 | 28.3 | 34.4 | 24.8 | 29.2 | 12.63 |
| ADF[2] | 27.1 | 20.9 | 20.3 | 21.4 | 22.6 | 21.3 | 19.9 | 21.1 | 18.7 | 23.4 | 25.7 | 27.1 | 18.7 | 22.1 | 11.40 |
| Tannins | 0.37 | 0.36 | 0.38 | 0.40 | 0.42 | 0.41 | 0.40 | 0.39 | 0.40 | 0.41 | 0.37 | 0.42 | 0.36 | 0.39 | 4.95 |
| Calcium | 0.30 | 0.40 | 0.38 | 0.41 | 0.45 | 0.31 | 0.38 | 0.41 | 0.34 | 0.34 | 0.35 | 0.45 | 0.30 | 0.37 | 12.50 |
| Phosphorous | 0.55 | 0.56 | 0.55 | 0.68 | 0.80 | 0.58 | 0.66 | 0.68 | 0.68 | 0.70 | 0.68 | 0.80 | 0.55 | 0.65 | 12.13 |
| Sulphur | 0.41 | 0.33 | 0.30 | 0.38 | 0.44 | 0.38 | 0.38 | 0.385 | 0.4 | 0.41 | 0.41 | 0.44 | 0.30 | 0.38 | 10.23 |
| Ash | 3.66 | 3.66 | 3.63 | 4.17 | 4.61 | 3.87 | 4.09 | 4.08 | 4.15 | 4.26 | 4.22 | 4.26 | 3.63 | 4.04 | 7.53 |
| Glucosinolate ($\mu$mole/g) | 11.72 | 8.98 | 7.02 | 8.69 | 12.90 | 10.52 | 13.08 | 10.65 | 12.59 | 10.71 | 10.91 | 13.1 | 7.0 | 10.7 | 17.66 |
| Gross energy (kcal/kg) | 6950 | 7102 | 7089 | 6958 | 7009 | 7126 | 6967 | 7030 | 6969 | 7040 | 6941 | 7126 | 6941 | 7016 | 0.94 |

[1]NDF = Neutral detergent fibre; [2]ADF = Acid detergent fibre;

[3]Samples 4, 7 and 8 were sourced from Ridley feedmill in Wasleys, SA
Samples 1, 2 and 3 were sourced from Cootamanudra in NSW
Samples 6 and 5 were provided by Dupond Pioneer in Westbrook, QLD.
Samples 9, 10 and 11 were soured from Ridley feedmill in Victoria.

Table 2 Analysed amino acids composition of canola seed samples (% of as is basis)

| AA profile | Canola seed samples | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Selected seeds for AME assay | | | | | | | | | | | | | | |
| | Seed 1 | Seed 2 | Seed 3 | Seed 4 | Seed 5 | Seed 6 | Seed 7 | Seed 8 | Seed 9 | Seed 10 | Seed 11 | Max | Min | Mean | CV % |
| *Indispensable AA* | | | | | | | | | | | | | | | |
| Arg | 1.35 | 1.16 | 1.01 | 1.29 | 1.46 | 1.17 | 1.30 | 1.27 | 1.32 | 1.36 | 1.39 | 1.46 | 1.01 | 1.28 | 9.80 |
| His | 0.70 | 0.65 | 0.53 | 0.67 | 0.73 | 0.65 | 0.70 | 0.67 | 0.68 | 0.70 | 0.70 | 0.73 | 0.53 | 0.67 | 7.84 |
| Ile | 0.95 | 0.84 | 0.73 | 0.91 | 0.99 | 0.85 | 0.91 | 0.91 | 0.91 | 0.96 | 0.97 | 0.99 | 0.73 | 0.90 | 8.17 |
| Leu | 1.59 | 1.39 | 1.21 | 1.53 | 1.70 | 1.41 | 1.54 | 1.52 | 1.56 | 1.62 | 1.65 | 1.7 | 1.21 | 1.52 | 9.10 |
| Lys | 1.51 | 1.31 | 1.14 | 1.41 | 1.56 | 1.37 | 1.41 | 1.39 | 1.47 | 1.51 | 1.52 | 1.56 | 1.14 | 1.42 | 8.41 |
| Avai Lys[1] | 1.48 | 1.29 | 1.12 | 1.39 | 1.54 | 1.35 | 1.39 | 1.37 | 1.45 | 1.49 | 1.50 | 1.54 | 1.12 | 1.40 | 8.49 |
| Met | 0.48 | 0.43 | 0.37 | 0.45 | 0.52 | 0.43 | 0.46 | 0.45 | 0.48 | 0.49 | 0.49 | 0.52 | 0.37 | 0.46 | 8.79 |
| M+C | 1.04 | 0.90 | 0.77 | 0.98 | 1.13 | 0.94 | 1.00 | 0.97 | 1.03 | 1.06 | 1.07 | 1.13 | 0.77 | 0.99 | 9.82 |
| Phe | 0.93 | 0.82 | 0.72 | 0.89 | 0.99 | 0.81 | 0.90 | 0.89 | 0.91 | 0.95 | 0.96 | 0.99 | 0.72 | 0.89 | 8.78 |
| Thr | 0.99 | 0.87 | 0.78 | 0.94 | 1.01 | 0.90 | 0.94 | 0.93 | 0.97 | 0.99 | 1.00 | 1.01 | 0.78 | 0.94 | 7.27 |
| *Dispensable AA* | | | | | | | | | | | | | | | |
| Ala | 0.97 | 0.84 | 0.74 | 0.92 | 1.01 | 0.86 | 0.93 | 0.91 | 0.95 | 0.98 | 0.99 | 1.01 | 0.74 | 0.92 | 8.61 |
| Asp | 1.56 | 1.36 | 1.24 | 1.49 | 1.65 | 1.33 | 1.50 | 1.47 | 1.51 | 1.56 | 1.59 | 1.65 | 1.24 | 1.48 | 8.28 |
| Cys | 0.56 | 0.48 | 0.41 | 0.53 | 0.62 | 0.51 | 0.54 | 0.52 | 0.56 | 0.57 | 0.58 | 0.62 | 0.41 | 0.53 | 10.46 |
| Glu | 3.65 | 3.19 | 2.61 | 3.50 | 4.01 | 3.21 | 3.58 | 3.50 | 3.61 | 3.70 | 3.77 | 4.01 | 2.61 | 3.48 | 10.68 |
| Gly | 1.08 | 0.95 | 0.83 | 1.04 | 1.15 | 0.97 | 1.04 | 1.03 | 1.07 | 1.10 | 1.12 | 1.15 | 0.83 | 1.03 | 8.72 |
| Pro | 1.35 | 1.09 | 0.96 | 1.25 | 1.49 | 1.17 | 1.23 | 1.20 | 1.32 | 1.35 | 1.37 | 1.49 | 0.96 | 1.25 | 11.73 |
| Ser | 0.90 | 0.81 | 0.70 | 0.87 | 0.94 | 0.82 | 0.88 | 0.86 | 0.90 | 0.91 | 0.92 | 0.94 | 0.70 | 0.86 | 7.82 |
| Trp | 0.24 | 0.22 | 0.27 | 0.24 | 0.32 | 0.27 | 0.29 | 0.28 | 0.24 | 0.30 | 0.33 | 0.33 | 0.22 | 0.27 | 13.02 |
| Tyr | 0.70 | 0.63 | 0.55 | 0.67 | 0.73 | 0.64 | 0.69 | 0.67 | 0.68 | 0.69 | 0.71 | 0.73 | 0.55 | 0.67 | 7.31 |
| Val | 1.19 | 1.04 | 0.91 | 1.14 | 1.24 | 1.06 | 1.15 | 1.13 | 1.15 | 1.20 | 1.22 | 1.24 | 0.91 | 1.13 | 8.44 |
| Total AA | 20.98 | 18.32 | 15.97 | 20.27 | 19.84 | 20.06 | 18.74 | 22.39 | 20.59 | 21.23 | 21.56 | 22.39 | 15.97 | 20.00 | 8.91 |

[1]Available Lysine, determined via carpenter assay: fluoro dinitro benzene reaction with epsilon amino group of lysine

Room temperature was gradually decreased from 33°C at first week to 24°C during 3rd week. From days 1 to 10 and 10 to 18 chicks had ad libitum access to conventional corn-soybean meal starter and grower *diets* respectively, to meet the nutrient specification of the strain as recommended by Ross 308 manual (2014).

***AME Assay Experimental Diets and Procedures***

Six CS samples differing in chemical composition (crude fat, crude fibre, crude protein and glucosinolate content) were selected from the eleven seeds assayed for nutrient composition. A common corn-soybean meal diet was formulated to serve as reference diet to meet or exceed the nutrient requirements of broiler chicks as described for the Ross 308 (2014). The six CS samples were incorporated into the reference diet at 15 % inclusion rate at the expense of corn, soybean meal and oil (85 % reference diet + 15 % CS) (Table 3). Titanium dioxide was added as an indigestible marker at 0.3 % of diet. All the diets were steam pelleted at temperatures of 75 or 90 ◦C. Accordingly, a 2 × 6 factorial arrangement of treatments was employed to investigate the effect of two aforementioned pelleting temperatures and six CS sources on ileal digestible energy (**IDE**), apparent metabolisable energy (**AME**) and apparent metabolisable energy corrected for nitrogen balance (**AMEn**) values of CS for grower broiler chickens. Accordingly, on 18 d of age, all the chicks were weighed and 420 chicks according to their mean body weight were randomly allocated to battery cages (80 cm× 45 cm× 50 cm) with 5 birds per cage and 6 replicate cages per dietary treatments. Birds were fed each of the 14 experimental diets (2 reference diet + 12 CS test diets) for 5 days as adaptation period followed by a 72-h energy balance assay from 22 to 25 d of age. Fresh water and feed were available to all chicks for *ad libitum* intake over the adaptation and collection period. During the 72-h collection period, feed consumption and excreta weights were recorded daily and used to calculate energy and nitrogen intake and excretion. Multiple subsamples were collected and homogenized from the total amount of excreta voided at the end of the collection period, and then a 250-g representative sample was placed in a plastic container for further analysis.

On d 25, all birds within cages were killed by $CO_2$ asphyxiation. Ileal contents (portion of the small intestine from Meckel's diverticulum to approximately 1 cm proximal to the ileo-caecal junction) were gently removed and pooled per replicate

cages, then frozen and stored at −20°C until processed. Representative samples of excreta and digesta were freeze-dried and finely ground.

Table 3 Ingredient and analysed nutrient composition of the experimental diets used for the AME assay.

| Ingredients % | Reference diet | CS[1] test diet 1 | CS test diet 2 | CS test diet 3 | CS test diet 4 | CS test diet 5 | CS test diet 6 |
|---|---|---|---|---|---|---|---|
| Corn (8.1 % CP) | 62.05 | 52.40 | 52.40 | 52.40 | 52.40 | 52.40 | 52.40 |
| Soybean meal (45.2 % CP) | 29.58 | 24.93 | 24.93 | 24.93 | 24.93 | 24.93 | 24.93 |
| Canola seed | - | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Canola oil | 3.59 | 2.86 | 2.86 | 2.86 | 2.86 | 2.86 | 2.86 |
| Limestone | 1.10 | 1.10 | 1.10 | 1.10 | 1.10 | 1.10 | 1.10 |
| Dicalcium Phosphate[2] | 1.62 | 1.62 | 1.62 | 1.62 | 1.62 | 1.62 | 1.62 |
| Sodium chloride | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Na Bicarbonate | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 |
| TiO2 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| VM premix[3] | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Choline Cl 70% | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| L-lysine HCl 78.4 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| DL-methionine | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 |
| L-threonine | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Analysed composition prior to pelleting %* | | | | | | | |
| Dry matter | 89.7 | 89.7 | 89.7 | 89.8 | 89.8 | 89.8 | 89.9 |
| Crude protein | 21.4 | 22.3 | 20.9 | 20.5 | 20.4 | 22.6 | 20.8 |
| Crude fat | 5.12 | 10.61 | 11.44 | 11.91 | 10.93 | 10.75 | 11.82 |
| Crude fibre | 2.13 | 2.90 | 2.93 | 2.86 | 2.92 | 2.55 | 2.66 |
| NDF | 8.03 | 12.18 | 9.81 | 12.37 | 11.44 | 10.17 | 9.33 |
| ADF | 3.80 | 8.42 | 8.71 | 8.63 | 8.27 | 9.12 | 6.72 |
| Ash | 5.83 | 6.70 | 6.53 | 6.57 | 6.81 | 6.70 | 6.31 |
| Calcium | 0.97 | 1.00 | 1.07 | 1.01 | 1.07 | 1.02 | 0.97 |
| Phosphorus | 0.58 | 0.68 | 0.65 | 0.64 | 0.70 | 0.75 | 0.61 |
| Sodium | 0.14 | 0.19 | 0.15 | 0.16 | 0.18 | 0.19 | 0.15 |
| Potassium | 0.90 | 0.88 | 0.85 | 0.83 | 0.91 | 0.88 | 0.88 |
| Sulphur | 0.27 | 0.33 | 0.31 | 0.31 | 0.34 | 0.33 | 0.31 |
| Chloride | 0.21 | 0.23 | 0.24 | 0.22 | 0.22 | 0.24 | 0.23 |

[1] CS: Canola seed

[2]Dicalcium phosphate contained: phosphorus, 18 %; calcium, 21 %.

[3]Trace mineral concentrate supplied per kilogram of diet: Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg. Vitamin concentrate supplied per kilogram of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg.

Table 4 Analysed chemical composition and pellet durability index (PDI) of the experimental diets after steam-pelleting at 75 º and 90 º C.

| Composition % | Reference diet | | CS[1] test diet 1 | | CS test diet 2 | | CS test diet 3 | | CS test diet 4 | | CS test diet 5 | | CS test diet 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 75 º C | 90 º C | 75 º C | 90 º C | 75 º C | 90 º C | 75 º C | 90 º C | 75 º C | 90 º C | 75 º C | 90 º C | 75 º C | 90 º C |
| DM | 89.8 | 88.4 | 89.0 | 88.8 | 88.7 | 88.4 | 89.0 | 88.7 | 88.9 | 88.3 | 89.0 | 88.3 | 88.9 | 88.5 |
| Crude protein | 21.7 | 21.6 | 21.9 | 22.1 | 20.8 | 20.6 | 20.8 | 20.4 | 20.8 | 20.9 | 22.7 | 22.4 | 20.8 | 20.5 |
| Crude fat | 5.2 | 5.6 | 10.9 | 11.1 | 11.4 | 11.5 | 12.4 | 12.1 | 10.8 | 10.9 | 10.8 | 10.6 | 11.8 | 11.5 |
| Crude fibre | 2.2 | 2.2 | 2.8 | 2.9 | 2.9 | 2.9 | 2.8 | 2.9 | 2.8 | 2.8 | 2.7 | 2.5 | 2.6 | 2.4 |
| NDF | 7.8 | 7.9 | 11.9 | 11.8 | 9.7 | 9.9 | 12.4 | 12.3 | 11.4 | 11.3 | 10.2 | 9.9 | 9.8 | 9.5 |
| ADF | 4.3 | 4.1 | 8.2 | 8.1 | 8.6 | 8.7 | 8.7 | 8.4 | 8.4 | 7.9 | 8.8 | 8.9 | 6.8 | 7.0 |
| Ash | 6.3 | 6.3 | 6.7 | 6.4 | 6.2 | 6.4 | 6.1 | 6.2 | 6.5 | 6.0 | 6.5 | 6.5 | 6.1 | 6.6 |
| Calcium | 1.03 | 1.00 | 1.03 | 1.05 | 1.07 | 1.05 | 0.99 | 0.99 | 1.04 | 1.05 | 1.03 | 1.05 | 0.98 | 1.00 |
| Phosphorous | 0.60 | 0.59 | 0.66 | 0.67 | 0.64 | 0.66 | 0.63 | 0.63 | 0.69 | 0.71 | 0.73 | 0.72 | 0.62 | 0.63 |
| Sodium | 0.16 | 0.14 | 0.15 | 0.17 | 0.14 | 0.16 | 0.14 | 0.17 | 0.16 | 0.17 | 0.17 | 0.15 | 0.16 | 0.16 |
| Potassium | 0.88 | 0.88 | 0.87 | 0.85 | 0.83 | 0.83 | 0.83 | 0.82 | 0.88 | 0.84 | 0.88 | 0.85 | 0.85 | 0.86 |
| Sulphur | 0.29 | 0.29 | 0.32 | 0.32 | 0.29 | 0.31 | 0.29 | 0.29 | 0.32 | 0.30 | 0.32 | 0.32 | 0.31 | 0.31 |
| Chloride | 0.22 | 0.23 | 0.24 | 0.23 | 0.22 | 0.21 | 0.22 | 0.23 | 0.23 | 0.21 | 0.22 | 0.24 | 0.23 | 0.23 |
| PDI | 88.4 | 95.2 | 83.4 | 88.2 | 82.3 | 88.0 | 79.5 | 84.6 | 85.7 | 89.1 | 86.4 | 91.2 | 86.1 | 89.8 |

[1] CS: Canola seed.

### *Pellet Durability Index*

Pellet quality was determined using a standardised method for durability (Method S269.4, ASAE, 1997) using Seedburo Pellet Durability Tester (developed by Kansas State University, USA). In this method, 500 g of sifted pellets were placed in a box and tumbled for 10 min at 50 rpm. The pellet durability index (**PDI**) was calculated as the percentage of pellets remaining after tumbling by dividing the weight of the whole pellets by 500 and multiplying by 100.

### *Chemical Analyses*

The diets, excreta, digesta and CS samples were analysed for DM by placing duplicate samples in a drying oven at 105°C for 24 h (method 930.15; AOAC, 1990). Gross energy content of feed, excreta, digesta and CS samples were determined on a 0.5 g sample using an adiabatic bomb calorimeter (IKA® Werke, C7000, GMBH and CO., Staufen, Germany) with benzoic acid as standard. Nitrogen content of feed, excreta, digesta and CS samples were determined on a 0.25 g sample with a combustion analyser (Leco model FP-2000 N analyser, Leco Corp., St. Joseph, MI) using EDTA as a calibration standard, with crude protein being calculated by multiplying percentage N by a correction factor (6.25). Titanium dioxide concentrations were determined in triplicate and duplicate for diets and digesta samples respectively, by colorimetric method as fully described by Short et al. (1995).

Chemical analyses [methods 920.39 (for crude fat), 982.30 E (for total lysine) 975.44 (for reactive lysine) 978.10 (for crude fibre), 973.18 (for neutral detergent fibre and acid detergent fibre), 942.05 (for ash), 927.02 (for calcium), 964.06 (for phosphorous), 990.31 (for sulphur)] of the CS samples were conducted according to AOAC (2006). The glucosinolate content of the meal was determined by colorimetric analyses using spectrophotometer according to the method described by Thies (1982) with the use of tetrachloropalladate as the highly specific reagent for glucosinolates.

***Calculations***

AME and AMEn of the reference and test diets were determined using the following equations:

AME (kcal/kg) = (GEI – GEE)/FI (Eq. 1)

AMEn (kcal/kg) = AME – [8.22 × (NI – NE)/FI] (Eq. 2)

Accordingly, the AMEn of the CS samples was calculated as:

CS AMEn kcal/kg (DM) = [Test diet AMEn – (reference AMEn x percentage of energy yielding ingredients in test diet relative to reference diet)] /percentage of inclusion rate (Eq. 3)

where GEI is the gross energy intake and GEE is the gross energy output of excreta (kcal/kg DM); 8.22 is nitrogen correction factor reported from previous research (Hill and Anderson, 1958); NI is nitrogen intake from the diet and NE is the nitrogen output from the excreta (kg); FI is the feed intake (kg).

Ileal digestible energy (IDE) of the reference and test diets was calculated using the following equations:

IE = GE in digesta × TiO2 in diet / TiO2 in digesta (Eq.4)

IDEC = [(diet gross energy – IE) ×100] / Diet gross energy (Eq.5)

where IE is ileal digesta gross energy content (kcal/kg DM) and IDEC is ileal digestibility of energy coefficient. Eq. 5 was used to calculate IDE of the CS samples.

***Statistical Analyses***

Performance data (derived from pen means), IDE, AME, and AMEn values were analysed as a 2×6 factorial arrangement using 2-way ANOVA of GLM procedure of SAS 9.2 package (2008) to assess the main effects (pelleting temperature and seed source) and 2-way interaction. Tukey's mean separation test was used to make pairwise comparisons between treatment means ($P < 0.05$). Pearson correlation coefficients and associated significance were generated using PROC GLM of SAS to

determine the relationship between seeds' analysed chemical compositions with AME, AMEn and IDE.

## RESULTS AND DISCUSSION

The nutrient composition and amino acid (**AA**) profile of CS samples are presented in Tables 1 and 2. The moisture content of the seed was rather consistent with an average of 5.7 % (CV % = 0.38). Crude protein and fat content ranged from 17.2 to 24.1 % and 40.8 to 47.9 %, respectively. The average crude fat of 42.9 % across CS samples was lower than the average values of around 47.9 % reported by Liu et al. (1995) and Assadi et al. (2011), but is within the same range of the crude fat levels reported for Australian CS (Seberry et al., 2013). The mean crude protein content of the CS observed in present study was slightly lower (21.5 % vs. 23.3 and 25.6 %) than those reported by Golian et al. (2007) and Najib and Al-Khateeb (2004), respectively. The gross energy content of the seeds exhibited small variability among the samples, with an average of 7016 kcal/kg with a CV % of 0.95, which are higher than the GE values previously determined for CS (Assadi et al., 2011). The glucosinolate concentrations of the samples had the greatest variability ranging from 7.0 to 13.1 µmole/g of seed (CV % = 17.66) and the mean value of 10.7 µmole/g glucosinolate of the samples is slightly higher than the values previously determined for Australian CS which is around 8.0 µmole/g (Seberry et al., 2013). The fibre content and composition varied considerably among samples with CV % of 7.24, 12.63 and 11.40 for crude fibre, NDF, and ADF, respectively. The average tannin concentration was around 0.39 % and its variation among samples was not considerably high (CV% = 4.95). The calcium, phosphorous and sulphur content of the seeds ranged from 0.30 to 0.45 %, 0.55 to 0.80 % and 0.30 to 0.44 %. Values of around 0.38 % of Ca and P have been reported for CS by Najib and Al-Khateeb (2004), while Assadi et al. (2011) determined a mean Ca and P content of 0.9 and 0.028%, respectively for three different varieties of CS. The authors attributed the large differences in Ca and P content of seeds to the agronomic and climatological conditions and the mineral content of the soil. Excluding the maximum and minimum values, the mean values of chemical composition of the samples ranged within the values reported for Australian canola seed (Seberry et al., 2013). As a function of crude protein, the total AA content of the seed samples ranged from 15.97 to 22.39

% with an average of 20.0 %. The average Met content (0.46 %) was the lowest relative to the other AA, whereas among indispensable AA, Leu and Lys contents were the highest in seed samples. The lowest and most abundant dispensable AA was Trp and Glu, respectively. The lowest variability in AA profile was observed for His, Thr, Tyr and Ser with an average CV of 7.5 % across the samples, while Trp and Pro had the greatest discrepancy among seed samples (CV % > 11.5).

The analysed chemical composition of the experimental diets prior to pelleting and post pelleting at 75 and 90°C are given in Tables 3 and 4, respectively. No noticeable differences of pellet temperature effect were observed on analysed composition of the diets and the values determined showed only negligible variations. The only consistent difference observed was the moisture content of feed which tended to increase after being conditioned with high temperature steam (90°C) during pelleting. The CS test diets had approximately 35.0 % higher crude fibre and 50.0 % higher crude fat compared to the reference diets. The total phosphorous level of CS test diets was also higher than the reference diet and corresponding to the phosphorous level of the seed, CS4 and CS5 test diets had the highest phosphorous content among the CS test diets. According to the data presented in Table 4, all the CS test diets had lower pellet durability index (**PDI**) compared to the corn-soybean reference diet. The CS test diet with the highest oil content in CS sample (sample 3) had the lowest PDI among the CS test diets. Increasing the pelleting temperature improved the PDI by approximately 5.0 percentage points across all the diets independent of seed source. In line with current findings, Cutlip et al. (2008) also reported that high conditioning temperature (93.3°C) during steam pelleting increased moisture content of the diets, decreased total fines by 2.5 percentage points and increased PDI and modified pellet durability index (MPDI) by 4.0 and 8.5 percentage points, respectively. Increased PDI and moisture content of the diet at higher temperature during steam pelleting, are likely because of increased moisture and heat that provided additional die lubrication and feed particle adhesion (Moritz et al., 2001, 2003).

Throughout the experiment, the mortality was below 1% and there were no signs of viral or bacterial infections. Bird performance is shown in Table 5. The BWG, daily feed intake, and FCR of the birds were not affected by differences in CS samples

and no interaction ($P > 0.05$) of seed source and pelleting temperature was detected for growth performance parameters. However, birds fed CS test diets had lower feed intake, BWG, and showed a lower FCR ($P < 0.05$) compared to birds fed the reference diet. Feeding a diet high in glucosinolate to broiler chicks has been reported to result in reduced feed intake, growth rate, and increased mortality (McNeill et al., 2004). However, glucosinolate levels of the CS test diets in the current study should not have exceeded 4 µmol/g of diet, which has been reported as the tolerance level for broiler chicks (Mawson et al., 1994). Apparently, the higher ME content of CS test diets, and the reduced palatability as a result of 15 % inclusion of CS, could have resulted in depressed feed intake and consequently weight gain.

Table 5 Effect of dietary treatments on performance of broilers from 18 to 25 d of age[1]

| Diet source | Pellet temperature | Initial BW (g) | Final BW (g) | BWG (g/b/d) | FI (g/b/d) | FCR (g/g) |
|---|---|---|---|---|---|---|
| Refer[2]diet | 75° C | 713 | 1367[a] | 93.4[a] | 127.4[a] | 1.364[a] |
| Refer diet | 90 ° C | 711 | 1356[a] | 92.1[b] | 125.9[b] | 1.366[a] |
| CS[3]diet 1 | 75° C | 712 | 1315[b] | 86.1[b] | 113.6[b] | 1.319[b] |
| CS diet 1 | 90 ° C | 713 | 1311[b] | 85.4[b] | 112.6[b] | 1.318[b] |
| CS diet 2 | 75° C | 712 | 1314[b] | 86.0[b] | 112.7[b] | 1.310[b] |
| CS diet 2 | 90 ° C | 711 | 1315[b] | 86.3[b] | 113.4[b] | 1.314[b] |
| CS diet 3 | 75° C | 715 | 1311[b] | 85.1[b] | 112.1[b] | 1.317[b] |
| CS diet 3 | 90 ° C | 714 | 1317[b] | 86.1[b] | 113.2[b] | 1.314[b] |
| CS diet 4 | 75° C | 714 | 1315[b] | 85.9[b] | 112.7[b] | 1.313[b] |
| CS diet 4 | 90 ° C | 714 | 1309[b] | 85.0[b] | 111.9[b] | 1.316[b] |
| CS diet 5 | 75° C | 714 | 1319[b] | 86.4[b] | 113.7[b] | 1.316[b] |
| CS diet 5 | 90 ° C | 713 | 1308[b] | 85.0[b] | 111.6[b] | 1.313[b] |
| CS diet 6 | 75° C | 712 | 1320[b] | 86.9[b] | 114.4[b] | 1.317[b] |
| CS diet 6 | 90 ° C | 715 | 1315[b] | 85.7[b] | 112.7[b] | 1.315[b] |
| | SEM | 4.24 | 19.67 | 2.26 | 3.48 | 0.023 |
| Source of variation (*P*-value) | | | | | | |
| Seed source | | 0.726 | 0.324 | 0.446 | 0.397 | 0.701 |
| Pellet temperature | | 0.844 | 0.199 | 0.214 | 0.273 | 0.824 |
| Source × temperature | | 0.926 | 0.462 | 0.337 | 0.765 | 0.637 |
| Refer diet vs. CS test diets | | 0.871 | 0.002 | 0.003 | 0.001 | 0.012 |

[a-b] Mean values within a column with no common letters are significantly different (Tukey test; $P < 0.05$)
[1]Data are means of 6 replicate cages with 5 broilers per cage
[2] Reference diet
[3] Canola seed test diet

Table 6 summarizes the effect of seed source and pelleting temperature on ileal digestible energy (IDE), AME, AMEn and gross energy (GE) retention of CS samples.

Table 6 Ileal digestible energy (IDE), AME, and $AME_n$ (kcal/kg DM) and retention of gross energy (%) of different sources of canola seed steam-pelleted at 75 or 90° C [1, 2].

| Seed source | IDE (kcal/kg) | AME (kcal/kg) | $AME_n$ (kcal/kg) | GE retention (%) |
|---|---|---|---|---|
| CS1 | 5239$^{e}$ | 4728$^{d}$ | 4501$^{c}$ | 64.7$^{c}$ |
| CS2 | 5579$^{ab}$ | 5071$^{a}$ | 4791$^{a}$ | 67.4$^{a}$ |
| CS3 | 5447$^{bc}$ | 5018$^{ab}$ | 4730$^{a}$ | 66.7$^{ab}$ |
| CS4 | 5427$^{cd}$ | 4819$^{dc}$ | 4554$^{bc}$ | 65.4$^{bc}$ |
| CS5 | 5298$^{de}$ | 4916$^{bc}$ | 4668$^{ab}$ | 66.6$^{ab}$ |
| CS6 | 5645$^{a}$ | 5018$^{ab}$ | 4740$^{a}$ | 66.5$^{ab}$ |
| SEM | 49.5 | 46.8 | 46.2 | 0.66 |
| | | | | |
| Pellet temperature | | | | |
| 75°C | 5495 | 4953 | 4682 | 66.5 |
| 90 °C | 5382 | 4903 | 4646 | 66.0 |
| SEM | 36.2 | 32.5 | 33.2 | 0.43 |
| | | | | |
| Source of variation (*P*-value) | | | | |
| Seed source | 0.001 | 0.001 | 0.002 | 0.011 |
| Pellet temperature | 0.061 | 0.203 | 0.357 | 0.204 |
| Source × temperature | 0.981 | 0.698 | 0.825 | 0.700 |

[1]Data are means of 6 replicate cages with 5 broilers per cage
[2]The AME and AMEn of the reference diets used to calculate the AME and AMEn of CS samples were (kcal/kg DM): 75ºC: 3,196, 3048; and 90ºC: 3,174, 3016;

There was no interaction of seed source and pelleting temperature for IDE, AME, AMEn and GE retention values of CS ($P > 0.05$). Pelleting temperature did not affect energy availability values of CS ($P > 0.05$). However, a significant effect of seed source was detected for all the energy utilization values of CS ($P < 0.05$). Theoretically, higher fat content of CS is expected to be associated with higher energy values. However, the impact of other chemical compositions particularly crude protein, fibre content and composition are usually overlooked. The IDE values across the CS samples ranged from 5,239 for CS1 (41.2, 16.5, and 22.5 % fat, fibre and protein content, respectively) to 5,645 kcal/kg DM for CS6 (45.8, 18.1, and 20.2 % fat, fibre and protein content, respectively). The IDE is part of the energy available to birds from a feed ingredient prior to microbial fermentation of energy substrates in the caeca and the relatively short colon (Adeola and Zhai, 2012). The IDE values of a feed ingredient are usually estimated to be greater than the corresponding AME values, because IDE just accounts for digested and absorbed energy in the gastrointestinal tract only up to the ileum and the energy containing compounds of

endogenous origin and urinary duct secreted post-ileum are not taken into the consideration. Irrespective of pelleting temperature all the IDE values calculated for the CS samples were all higher than AME values, with the highest difference being 11.3 % for CS6 and the lowest difference 7.22 % for CS5.

Measurement of IDE followed a similar trend with CS1 having lowest in AME and AMEn being 4,728 and 4,501 kcal/kg DM, respectively. But, the highest AME and AMEn (5,071 and 4,791 kcal/kg DM, respectively) values were observed for CS2. This seed sample, despite a high level of fat (45.9 %) had the lowest fibre and NDF content compared to the other CS samples. Despite the high fat content of CS3 (47.9%) compared to the other samples, it did not necessarily have the highest energy availability values. This could likely be due to its lower crude protein, higher fibre and NDF content, since fibre is poorly digested by poultry and affect energy utilisation values of an ingredient by accelerating the digesta passage rate, which in turn, may result in reduced time for digestion and thus reduced nutrient utilization (Bell et al., 1991; Khajali and Slominski, 2012). Adeola and Ileleji (2009) also estimated 589 kcal/kg higher AME in corn distillers grains with solubles which had 50 and 45 % lower concentrations of NDF and ADF, respectively, than corn distillers grains without solubles. Similar to the results of the current study, Assadi et al. (2011) indicated that heat-treatment did not have any significant effect on AME and AMEn values of CS. The authors also showed that higher fat content of CS was not necessarily associated with higher AMEn values, and speculated that NDF and ADF content of the seed negatively affect energy utilisation values of CS. Meng et al. (2006) observed more than 1,000 kcal/kg DM improvement in CS TMEn by using a combination of carbohydrase. They showed that this improvement in energy availability with enzyme supplementation was directly related to improved fat digestibility ($r^2$ = 0.94; P < 0.0001) which was highly correlated with improved NSP digestibility ($r^2$ = 0.84; P < 0.0001). These relationships between carbohydrate and fat digestibility and improved energy utilisation of the CS, could signify the nutrient-encapsulating effect of cell walls in CS and the impact of its fibre content of seed on energy availability for the birds.

Retained nitrogen in the body yields energy-containing compounds with metabolites that are voided in the urine; therefore, AME values are corrected to zero nitrogen

balance to adjust for the effect of differences in protein retention across birds in any assay in order to reduce the variability in estimates of AME (Leeson et al., 1977; Lopez and Leeson, 2007). Correcting the AME values for nitrogen retention decreased the values by approximately 5.7 % from the corresponding AME values across all the CS samples. Apparently, as the test diets were not deficient in nitrogen and bird's need for protein was met, catabolism of body protein was reduced resulting in a positive nitrogen balance. Reductions in the range of 4 to 10% have been reported in several studies with broilers and ducks (Hong et al., 2002; Adeola et al., 2007; Adeola and Ileleji, 2009). The mean AMEn values of 4,664 kcal/kg DM obtained in this study are comparable with the values of 4,460 and 4,442 as reported by Lee et al. (1995) and Assadi et al. (2011) but slightly lower than a sample previously measured by Barekatain et al. (2015) as 4691 kcal/kg DM.

Pearson correlations between energy utilization and chemical components of CS are presented in Table 7. Crude protein content of the CS was negatively correlated to fat content of the samples (r = -0.93; P = 0.001), and gross energy (r = -0.66; P = 0.001), but positively correlated to sulphur concentration (r = 0.95; P = 0.001). Consequently, IDE, AME and AMEn values were also negatively correlated to protein content (r = -0.40, -0.39, and -0.32; respectively, P = 0.009). There was a positive correlation between crude fat of the CS samples and GE (r = 0.87), IDE (r = 0.53), AME (r = 0.712) and AMEn (r = 0.65) at P = 0.001. No strong significant correlation between crude fibre and energy utilisation values of CS was detected, but ADF was negatively correlated to GE, IDE, AME and AMEn (approx. r = -0.50; P = 0.001). Glucosinolate was positively correlated to sulphur (r = 0.86; P = 0.001), crude fibre and NDF (r = 0.43; P = 0.001), but there was no significant correlation to AME or AMEn values. Indeed, more than one chemical component in a feed ingredient and the interactions between them may influence AMEn values. Generally, it is accepted that fibre content and composition and crude fat may have the greatest impact on AMEn content of CS. However, as the samples varied in chemical composition, the correlations obtained between crude fibre and fat with energy utilisation values were not very strong.

Table 7 Pearson correlation coefficients between processing chemical components and energy utilization of canola seed samples[1]

| Item | Gross energy | IDE | AME | $AME_n$ | GE retention | Crude protein | Crude fat | Crude fibre | NDF | ADF | Tannins | Glucos-inolate | Sulphur |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gross energy | 1.00 | | | | | | | | | | | | |
| *P*-value | - | | | | | | | | | | | | |
| IDE | 0.545 | 1.00 | | | | | | | | | | | |
| *P*-value | 0.001 | - | | | | | | | | | | | |
| AME | 0.581 | 0.292 | 1.00 | | | | | | | | | | |
| *P*-value | 0.001 | 0.012 | - | | | | | | | | | | |
| $AME_n$ | 0.527 | 0.232 | 0.981 | 1.00 | | | | | | | | | |
| *P*-value | 0.001 | 0.049 | 0.001 | - | | | | | | | | | |
| GE retention | 0.387 | 0.181 | 0.975 | 0.979 | 1.00 | | | | | | | | |
| *P*-value | 0.001 | 0.127 | 0.001 | 0.001 | - | | | | | | | | |
| Crude protein | -0.661 | -0.406 | -0.389 | -0.321 | -0.261 | 1.00 | | | | | | | |
| *P*-value | 0.001 | 0.004 | 0.009 | 0.006 | 0.026 | - | | | | | | | |
| Crude fat | 0.877 | 0.532 | 0.715 | 0.649 | 0.591 | -0.930 | 1.00 | | | | | | |
| *P*-value | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | - | | | | | | |
| Crude fibre | 0.414 | 0.168 | 0.129 | 0.113 | 0.035 | -0.133 | 0.317 | 1.00 | | | | | |
| *P*-value | 0.003 | 0.156 | 0.277 | 0.342 | 0.769 | 0.263 | 0.006 | - | | | | | |
| NDF | -0.221 | -0.329 | -0.176 | -0.173 | -0.140 | -0.280 | 0.139 | 0.234 | 1.00 | | | | |
| *P*-value | 0.062 | 0.004 | 0.137 | 0.144 | 0.238 | 0.017 | 0.243 | 0.047 | - | | | | |
| ADF | -0.669 | -0.472 | -0.497 | -0.422 | -0.382 | 0.614 | -0.715 | -0.165 | 0.236 | 1.00 | | | |
| *P*-value | 0.001 | 0.001 | 0.001 | 0.002 | 0.009 | 0.001 | 0.001 | 0.165 | 0.045 | - | | | |
| Tannins | 0.006 | 0.030 | 0.025 | 0.027 | 0.029 | 0.478 | -0.255 | 0.525 | -0.325 | -0.254 | 1.00 | | |
| *P*-value | 0.959 | 0.802 | 0.831 | 0.817 | 0.803 | 0.001 | 0.030 | 0.001 | 0.005 | 0.030 | - | | |
| Glucosinolate | 0.082 | -0.289 | -0.123 | 0.038 | -0.022 | 0.234 | -0.033 | 0.437 | 0.435 | 0.350 | 0.137 | 1.00 | |
| *P*-value | 0.489 | 0.012 | 0.845 | 0.747 | 0.812 | 0.047 | 0.782 | 0.001 | 0.001 | 0.002 | 0.249 | - | |
| Sulphur | 0.025 | -0.154 | -0.261 | 0.085 | 0.412 | 0.952 | -0.784 | 0.398 | 0.066 | 0.311 | 0.321 | 0.863 | 1.00 |
| *P*-value | 0.561 | 0.115 | 0.216 | 0.561 | 0.841 | 0.001 | 0.008 | 0.012 | 0.419 | 0.025 | 0.045 | 0.001 | - |

[1] IDE = Ileal digestible energy; AME = Apparent metabolisable energy; $AME_n$ = Nitrogen corrected AME; GE retention: gross energy retention of seed; NDF = Neutral detergent fibre; ADF = Acid detergent fibre;

## CONCLUSION

In conclusion, the present study demonstrates that chemical composition of canola seed has a significant effect on its energy availability for broiler chickens. Therefore, nutritionists should be cautious of the source of data for AMEn values of CS when formulating diets to reduce feed costs and also improve bird performance. Increasing pellet temperature during steam pelleting up to 90°C was not effective in releasing more energy from CS, but improved pellet durability index of the diets.

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# Chapter 3 Interactions of full fat canola seed, oat hulls as an insoluble fibre source and pellet temperature for nutrient utilisation and growth performance of broiler chickens

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## ABSTRACT

The effectiveness of the addition of oat hulls (OH) as an insoluble fibre for improving nutrient digestibility and performance of birds fed diets containing full fat canola seed (CS) was studied. A 2 × 2 × 2 factorial arrangement of treatments was used assessing the main effects of canola source (canola seed vs canola meal plus oil as control), oat hulls (0 or 30 g/kg), pellet temperature (75 and 90°C) and their interactions. A total of 576 male day-old Ross 308 chickens were assigned to 8 experimental treatments, each replicated 6 times (12 birds per replicate). All birds were fed the same commercial starter diet for the first 10 d of age. Canola meal and canola oil in the control diets were replaced with CS at 116 g/kg and 135 g/kg in the grower (d 10-24) and finisher (d 24-35) diets, respectively. A significant interaction was observed between canola source and OH led to improved body weight gain ($P < 0.01$) and FCR ($P < 0.05$) in birds fed the combination of CS and OH in grower phase of feeding. Pelleting temperature at 75 vs 90°C did not affect performance of broilers. Birds fed diets containing OH had heavier gizzards at 24 and 35 d of age. Inclusion of CS in the diets depressed fat digestibility at d 24 ($P < 0.001$) and AME of the grower diets. At d 35, there was a significant interaction ($P < 0.05$) between CS and pellet temperature where birds fed CS diets pelleted at 75°C had higher fat digestibility than birds fed CS pelleted at 90°C. Regardless of canola or pellet temperature, OH increased fat utilization at d 35 ($P < 0.001$) but had no effect on AME of the grower diets. In conclusion, CS can replace supplemental oil in broiler diets when an adequate source of insoluble fibre is included in diet which may help to maintain feed intake broilers fed CS in steam-pelleted diets.

**Key words:** Insoluble fibre, gut development, fat digestibility, amino acids

## INTRODUCTION

Whole canola seed (CS), due to its high oil content, is a high energy ingredient that can potentially replace a substantial proportion of supplemental oil in poultry diet. However, the high level inclusion of CS is poultry diet are often avoided due to concerns over residual glucosinolates and adverse effect on feed consumption (Barekatain, et al., 2015; Summers, et al., 1982). The results of a recent experiment showed that despite a desirable outcome in bird performance of broiler diets containing CS, these diets were not fully utilised when steam pelleted and therefore feed consumption and body weight was adversely affected (Barekatain, et al., 2015). In addition, pre-grinding of the seed did not improve fat utilisation or bird performance when diets were cold-pelleted but there was marginal improvement in performance when the diet was steam-pelleted. It was hypothesized that pellet temperature and differences in seed myrosinase and glucosinolate levels are determinants of CS utilisation, particularly with respect to feed intake. The degree to which pelleting temperature can affect the nutritive values of CS is not fully understood. Furthermore canola contains the enzyme myrosinase that catalyses the breakdown of glucosinolate to other metabolites including isothiocyanate which is a bitter compound that can be minimised by applying heat to denature the myrosinase enzyme (Tripathi and Mishra, 2007).

The reduced fat digestibility in canola seed diets has been attributed to fat in seeds being contained in oil bodies that are surrounded by a peptide coating that may limit digestibility of the oil (Meng, et al., 2006). Ways of breaking the peptide coating could include milling of the whole CS prior to diet addition (Barekatain, et al., 2015), use of exogenous enzymes (Slominski, et al., 2006), or possibly addition of insoluble fibre to stimulate gizzard activity which builds on results for beneficial effect of oat hulls in broiler diets. If fat digestibility is increased by addition of OH, then this is likely to lead to benefits when oil seeds are used commercially to replace supplemental oil in poultry diets.

The beneficial impact of inclusion of insoluble fibre in the diet of broiler chickens has been well demonstrated in literature. Studies conducted with broilers have shown that moderate amounts of dietary fibre result in significant improvement in utilisation by birds of most nutrients (Amerah, et al., 2009; Mateos, et al., 2012). In addition, Gonzalez-Alvarado et al. (Gonzalez-Alvarado, et al., 2007) and Kalmendal et al.

(2011) showed an increase in fat digestibility when fibre was included in broiler diets. However, the interaction between insoluble fibre sources (e.g. OH) with utilisation of oil seeds such as full fat CS has not been investigated. When it is assumed that fibre results in a more developed gizzard and intestine of the birds, it is hypothesised that there is increased scope for improved nutrient intake through nutrient release and lower carbohydrate solubilisation in wheat-based diets which contain CS. If this hypothesis is proven successful, one can conclude that CS can be used in lieu of supplemental oil in broiler diets without compromising feed intake or body weight. Furthermore, there is no need for pre-grinding which otherwise could be cumbersome and costly due to seed size and high oil content. Thus, it was proposed to investigate the effect of OH as a rich source of insoluble fibre, CS and pelleting temperature on performance, intestinal development and nutrient utilisation.

## MATERIALS AND METHODS

The Animal Ethics Committees of the University of Adelaide and Primary Industries and Regions South Australia approved all the experimental procedures.

### *Ingredient analysis*

Chemical analysis of CS and canola meal are shown in Table 8. Due to timing limitations of the experiments, it was not possible to use the same seed as used in Chapter 2. Therefore, the AME value of 20.24 MJ/kg was used for the CS which was the average of the values reported in the literature (from 18.42 to 22.06 MJ/kg, Barekatain et al. (2015). Wheat and sorghum were also analysed prior to feed formulation. All samples were analysed in duplicate using AOAC (2005) methods of 920.39 for crude fat, 978.10 for crude fibre, and 942.05 for ash.

### *Experimental design and diets*

A 2 × 2 × 2 factorial arrangement of treatments was used to investigate three main factors of CS inclusion (CS vs canola meal), oat hulls inclusion (0 and 3%) and pellet temperature (75°C or 90°C). Canola seed was added as whole when diets were mixed. Canola meal and oil in control diets were replaced with CS. Diets were

calculated to be isoenergetic and isonitrogenous. In line to the level of canola meal and oil in control diets, inclusion rate of CS was 116 g/kg and 135 g/kg for grower (d 10-24) and finisher (d 24-35) diets, respectively (Table 9). All diets were formulated to meet the requirements of Ross 308 broiler chickens (Ross, 2014).

Table 8 Chemical analysis (%) of canola meal and full-fat canola seed (as is basis)[1]

| Item | Canola meal | Full-fat canola seed |
|---|---|---|
| Moisture | 10.10 | 5.73 |
| Crude protein | 38.23 | 27.35 |
| Crude fibre | 9.90 | 7.97 |
| Crude fat | 2.41 | 35.20 |
| Ash | 6.87 | 4.79 |
| | | |
| Indispensable amino acids | | |
| Arginine | 1.67 | 2.24 |
| Cysteine | 0.71 | 0.92 |
| Histidine | 0.74 | 1.00 |
| Isoleucine | 1.01 | 1.40 |
| Leucine | 1.88 | 2.69 |
| Lysine | 1.69 | 2.20 |
| Methionine | 0.55 | 0.75 |
| Phenylalanine | 1.13 | 1.53 |
| Threonine | 1.14 | 1.66 |
| Tryptophan | 0.28 | 0.47 |
| Valine | 1.31 | 1.83 |
| | | |
| Dispensable amino acids | | |
| Alanine | 1.15 | 1.66 |
| Aspartic Acid | 1.91 | 2.61 |
| Glutamic Acid | 4.72 | 6.89 |
| Glycine | 1.34 | 1.93 |
| Proline | 1.64 | 2.26 |
| Serine | 1.09 | 1.60 |
| Tyrosine | 0.68 | 0.96 |

[1]Values are the mean of duplicate samples

Table 9 Ingredient, nutrient composition (calculated and/or measured) and pellet durability index of experimental grower and finisher diets

| | Grower | | | | Finisher | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Control + Oat hulls | Canola seed | Canola seed + oat hulls | Control | Control + Oat hulls | Canola seed | Canola seed + oat hulls |
| Ingredient (%) | | | | | | | | |
| Sorghum | 22.000 | 22.000 | 19.180 | 22.000 | 22.000 | 22.000 | 22.000 | 22.000 |
| Wheat | 38.216 | 33.776 | 39.898 | 32.589 | 40.633 | 36.193 | 38.896 | 34.438 |
| Oat hulls | 0.000 | 3.000 | 0.000 | 3.000 | 0.000 | 3.000 | 0.000 | 3.000 |
| Soybean meal | 24.802 | 25.592 | 25.249 | 26.121 | 22.103 | 22.893 | 21.919 | 22.735 |
| Canola seed | 0.000 | 0.000 | 11.600 | 11.600 | 0.000 | 0.000 | 13.500 | 13.500 |
| Canola meal solvent | 6.500 | 6.500 | 0.000 | 0.000 | 6.500 | 6.500 | 0.000 | 0.000 |
| Canola Oil | 4.320 | 5.150 | 0.000 | 0.784 | 4.986 | 5.816 | 0.000 | 0.834 |
| Limestone | 1.132 | 1.017 | 1.147 | 1.031 | 1.096 | 0.980 | 1.105 | 0.990 |
| Dicalcium phosphate | 1.144 | 1.083 | 1.147 | 1.093 | 1.002 | 0.940 | 1.002 | 0.941 |
| Xylanase powder 50g/mt | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 |
| Phytase 5000 U/g | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 |
| Sodium chloride | 0.153 | 0.154 | 0.173 | 0.174 | 0.179 | 0.181 | 0.198 | 0.203 |
| Sodium bicarbonate | 0.306 | 0.299 | 0.292 | 0.290 | 0.268 | 0.262 | 0.261 | 0.250 |
| $TiO_2$ | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 |
| Vitamin and mineral Premix[3] | 0.200 | 0.200 | 0.200 | 0.200 | 0.200 | 0.200 | 0.200 | 0.200 |
| Choline Cl 70% | 0.124 | 0.130 | 0.047 | 0.055 | 0.131 | 0.137 | 0.046 | 0.052 |
| L-lysine HCl 78.4 | 0.208 | 0.197 | 0.193 | 0.183 | 0.120 | 0.108 | 0.117 | 0.095 |
| DL-methionine | 0.262 | 0.269 | 0.251 | 0.260 | 0.200 | 0.207 | 0.186 | 0.192 |
| L-threonine | 0.118 | 0.118 | 0.107 | 0.107 | 0.068 | 0.068 | 0.056 | 0.055 |
| Nutrient composition (% unless otherwise specified) | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Metabolisable energy | kcal/kg | 3038 | 3038 | 3038 | 3038 | 3100 | 3100 | 3100 | 3100 |
| Crude Protein | | 21 | 21 | 21 | 21 | 19.9 | 19.9 | 19.9 | 19.9 |
| Crude protein (measured) | | 21.16 | 20.89 | 21.70 | 21.67 | 20.62 | 20.80 | 20.53 | 20.36 |
| Crude fat (measured) | | 6.15 | 7.19 | 7.41 | 7.26 | 6.47 | 6.86 | 6.52 | 7.68 |
| Crude fibre (measured) | | 2.56 | 3.43 | 2.55 | 3.42 | 2.62 | 3.53 | 2.67 | 3.58 |
| ADF (measured) | | 4.87 | 6.02 | 5.89 | 5.61 | 4.65 | 5.49 | 5.32 | 6.51 |
| NDF (measured) | | 8.43 | 10.50 | 8.36 | 9.78 | 9.07 | 10.87 | 8.99 | 9.77 |
| Dig Arg | | 1.23 | 1.24 | 1.24 | 1.24 | 1.16 | 1.16 | 1.16 | 1.16 |
| Dig Lys | | 1.1 | 1.1 | 1.1 | 1.1 | 0.97 | 0.97 | 0.977 | 0.97 |
| Dig Met | | 0.55 | 0.55 | 0.53 | 0.54 | 0.47 | 0.48 | 0.46 | 0.47 |
| Dig Cys | | 0.29 | 0.28 | 0.29 | 0.28 | 0.28 | 0.27 | 0.28 | 0.28 |
| Dig M+C | | 0.84 | 0.84 | 0.84 | 0.84 | 0.76 | 0.76 | 0.76 | 0.76 |
| Dig Trp | | 0.24 | 0.24 | 0.24 | 0.24 | 0.22 | 0.22 | 0.22 | 0.22 |
| Dig Leu | | 1.39 | 1.39 | 1.37 | 1.39 | 1.32 | 1.33 | 1.32 | 1.32 |
| Dig Ile | | 0.84 | 0.84 | 0.84 | 0.84 | 0.80 | 0.80 | 0.80 | 0.80 |
| Dig Thr | | 0.73 | 0.73 | 0.73 | 0.73 | 0.65 | 0.65 | 0.65 | 0.65 |
| Dig Val | | 0.93 | 0.93 | 0.93 | 0.93 | 0.88 | 0.88 | 0.88 | 0.88 |
| Calcium | | 0.90 (0.92) | 0.90 (0.94) | 0.90 (0.89) | 0.90 (0.87) | 0.85 (0.86) | 0.85 (0.83) | 0.85 (0.81) | 0.85 (0.92) |
| Phosphorus (available) | | 0.45 | 0.45 | 0.45 | 0.45 | 0.42 | 0.42 | 0.42 | 0.42 |
| Phosphorus total (measured) | | 0.66 | 0.62 | 0.64 | 0.65 | 0.61 | 0.61 | 0.63 | 0.64 |
| Sodium | | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Chloride | | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| PDI[4] | | 89 | 84 | 94.6 | 90.4 | 88.6 | 84.2 | 92.6 | 88.7 |

[3]Trace mineral concentrate supplied per kilogram of diet: Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg. Vitamin concentrate supplied per kilogram of diet:

retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg.

[4] PDI: Pellet durability index was determined using a standardised method for durability (Method S269.4, ASAE, 1997) using Seedburo Pellet Durability Tester (developed by Kansas State University, USA).

The diets were steam-pelleted at two different temperature of 75 and 90°C at The University of Sydney, Camden, NSW similar to the procedures previously described by Selle, et al. (2013). A Palmer PP300 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) was used to steam-pellet the experimental diets. The die dimensions were 4-mm diameter and 45-mm length. The conditioning temperature was being automatically regulated via a computer software package (Gordyn & Palmer, Hallam, Vic, Australia) equipped to the pellet press (Selle, et al., 2013). The residence time in the conditioner was 30 seconds.

***Housing and bird management***

A total of 576 male day-old Ross 308 broiler chickens were obtained (Baiada hatchery, Willaston, South Australia). All birds were raised on a floor based pen receiving a same commercial diet from d 0 to d 10. On d 10, birds were assigned to 48 rearing pens on wood shavings in a temperature-controlled system. Each treatment was replicated 6 times with each replicate accommodating 12 birds. Temperature was set at 33-34ºC on the first day of the experiment and then gradually decreased by 1ºC every second day until a stable temperature of 24ºC was reached by d 21. In the first week of age, birds had 23 h light and 1 h dark. Subsequently, birds received 16 h light and 8 h dark. Feed and water were provided ad libitum throughout the experiment. At the end of each phase of feeding, birds were weighed and feed consumption and FCR were measured. FCR, was adjusted morality if occurred for any treatment.

***Sample collection, viscosity measurement and nutrient digestibility assay***

On d 24 and 35, three birds per replicate were randomly chosen and euthanized in order to obtain ileal digesta and record visceral organ weights. The empty weight of duodenum, jejunum, ileum, proventriculus, and gizzard were expressed to body weight. The ileal contents of three birds were collected into ice-cold plastic containers, and subsequently pooled by each replicate pen. To measure the viscosity of ileal content 9 (only on d 35), subsamples were immediately centrifuged (12000×g, 10 min, 4°C) to obtained supernatant. About 0.5 mL of thawed

supernatant was used to measure viscosity with a Brookfield DVIII viscometer at 25°C with a CP 40 cone. The shear rate was from 5 to 500/s. The remaining samples were stored at -20°C and then freeze-dried before conducting further analyses.

The methods of AOAC (2005) were followed for determination of dry matter (DM) and fat content of diets and digesta. The N content analysis of the diets and digesta samples was performed using a LECO FP-2000 automatic analyser (Leco Corporation, MI, USA). Amino acid determination was conducted by hydrolysing the samples with 6M HCl (containing phenol) for 24 h at 110 ± 2°C in glass tubes sealed under vacuum conducted by Macquarie University, NSW, Australia. Titanium contents of diets and ileal samples were measured using the method fully described by Short et al. (1996). Subsequent digestibility coefficients for amino acids, N, DM and fat were calculated as follows:

Apparent ileal digestibility coefficient:

$$= \frac{(NT/Ti)_d - (NT/Ti)_i}{(NT/Ti)_d}$$

where,

$(NT/Ti)_d$ was the ratio of nutrient (NT) and titanium (Ti) in diet

$(NT/Ti)_i$ was the ratio of nutrient (NT) and titanium (Ti) in ileal digesta

### *Apparent metabolisable energy*

Apparent metabolisable energy (AME) bioassay was conducted for grower diets using total collection method. A total of 192 Ross 308 broiler chickens from same batch of birds used in the feeding study, were separately raised on a floor based pen with wood shavings as litter from day 1 to d 17 of age. All bird received a same commercial diet and had access to feed and water *ad libitum*. On d 18, birds were transferred to a total of 48 metabolism cages each accommodating 4 birds and were given eight experimental treatments used in feeding trial after two days of adaptation period. Total excreta collection was conducted over four consecutive days. All collected excreta was oven dried at 85°C, weighed and then finely ground for gross energy analysis.

The gross energy (GE) content of experimental diets and excreta were determined using a Parr isoperibol bomb calorimeter (Parr Instrument Company, Moline, IL) with benzoic acid as the standard.

The AME of the experimental diets were determined using the following equation:

AME (MJ/kg) = (GEI-GEE)/FI

where GEI is gross energy intake and GEE is gross energy output of excreta (MJ/kg of DM); and FI is the feed intake (kg).

### *Statistical analysis*

Data of the experiment were subjected to statistical analysis using 3-way ANOVA of GLM procedure of SAS (2003) according to a 2×2×2 factorial arrangement of treatments to assess the main effects of canola inclusion, oat hulls, pellet temperature and their 2- or 3-way interactions. Data were checked for normal distribution. One cage constituted an experimental unit and the values presented in the tables are means with pooled standard error of mean (SEM) (n=48). If a significant effect was detected, differences between treatments or main effects were separated by Duncan multiple range test. All statements of significance are considered on a *P*-value less than 0.05 and tendencies were specified for $0.05 < P < 0.10$.

## RESULTS

### *Growth performance*

The results of feed intake, BW gain and FCR of birds are given in Table 10. There were no three way interactions for any of performance parameters. Feed consumption tended ($P = 0.088$) to decrease in birds fed CS from d 10-24. At the same time, there was a tendency ($P = 0.078$) for higher FI when OH were included in the diets. For both grower and finisher phases, there was a significant interaction ($P < 0.05$) between OH and type of canola where birds fed both CS and OH had a higher FI than CS alone. Pellet temperature did not influence performance of broiler at any stage of the experiment.

Canola seed and OH interacted significantly resulting in a higher BW gain of birds from d 10 to 24 of age. Inclusion of OH independently improved BW gain from d 24 to 35 and when assessed for entire study. By a significant interaction, FCR was improved in birds offered OH and CS in both grower ($P$<0.05) and finisher ($P$<0.01) phase of feeding.

***Organ weights***

The relative weights of organs are presented in Table 11. Dietary treatments did not influence the relative weight of proventriculus at d 24 or 35. There was significant interaction between OH inclusion and pellet temperature for relative gizzard weight where higher pellet temperature increased gizzard weight only in absence of OH in the diet ($P$ < 0.05). Addition of OH to the diets independently increased gizzard weight at both d 24 and d 35 ($P$ < 0.001) and ileum weight ($P$ < 0.05) at d 35. Canola seed inclusion and OH tended to interact for duodenal weight where birds fed diets containing CS and OH had heavier duodenum ($P$ = 0.07). Rising temperature to 90°C increased relative weight of duodenum at d 24 ($P$ < 0.05) and d 35 ($P$ < 0.01).
On d 24, birds fed CS had heavier jejunum than birds receiving diets containing CM and oil ($P$ < 0.05).

***Ileal nutrient digestibility***

The results of ileal DM, N and fat digestibility measurements are given in Table 12. At d 24, there was an interaction between the type of canola inclusion and pellet temperature for DM digestibility ($P$ < 0.01) when birds fed CS diets pelleted at 75°C had lower DM digestibility. There was no interaction between any of the three experimental factors for DM digestibility at d 35. At the same time, inclusion of either OH, CS or pelleting temperature at 90°C independently reduced DM utilization ($P$ < 0.05).
There was no interaction between the experimental factors for ileal digestibility of N on either d 24 or 35. At d 24, feeding CS tended to decrease N digestibility ($P$ = 0.067). For both grower and finisher diets, birds fed OH had higher N digestibility coefficient compared to birds fed OH-free diets ($P$ < 0.05).

Table 10 Feed intake, body weight gain and feed conversion ratio of broiler chicken fed experimental grower and finisher diets

| Treatment | | | Feed intake (g/bird) | | | Body weight gain (g/bird) | | | Feed conversion ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oat hulls (%) | Temperature | 10-24 | 24-35 | 10-35 | 10-24 | 24-35 | 10-35 | 10-24 | 24-35 | 10-35 |
| Canola meal | 0 | 75$^0$C | 1462 | 2086 | 3548 | 1036 | 1270 | 2306 | 1.415$^{a}$ | 1.644$^{a}$ | 1.540 |
| Canola meal | 0 | 90$^0$C | 1450 | 2081 | 3530 | 1021 | 1293 | 2314 | 1.420$^{a}$ | 1.610$^{ab}$ | 1.526 |
| Canola meal | 3 | 75$^0$C | 1474 | 2068 | 3542 | 1023 | 1344 | 2367 | 1.442$^{a}$ | 1.540$^{c}$ | 1.496 |
| Canola meal | 3 | 90$^0$C | 1451 | 2107 | 3558 | 1029 | 1324 | 2354 | 1.411$^{ab}$ | 1.592$^{abc}$ | 1.512 |
| Canola seed | 0 | 75$^0$C | 1410 | 1997 | 3407 | 1001 | 1283 | 2285 | 1.408$^{ab}$ | 1.561$^{bc}$ | 1.496 |
| Canola seed | 0 | 90$^0$C | 1416 | 2058 | 3474 | 1042 | 1305 | 2347 | 1.360$^{bc}$ | 1.578$^{bc}$ | 1.481 |
| Canola seed | 3 | 75$^0$C | 1445 | 2141 | 3587 | 1096 | 1355 | 2452 | 1.319$^{c}$ | 1.580$^{bc}$ | 1.464 |
| Canola seed | 3 | 90$^0$C | 1454 | 2101 | 3556 | 1082 | 1329 | 2411 | 1.345$^{c}$ | 1.581$^{bc}$ | 1.475 |
| | | SEM | 6.02 | 9.90 | 12.81 | 5.97 | 8.00 | 11.13 | 0.0066 | 0.0066 | 0.0045 |
| *Main effects* | | | | | | | | | | | |
| Canola | | Meal | 1458 | 2085 | 3544 | 1027 | 1308 | 2335 | 1.422 | 1.596 | 1.518$^{a}$ |
| | | Seed | 1431 | 2074 | 3505 | 1055 | 1318 | 2372 | 1.359 | 1.575 | 1.478$^{b}$ |
| Oat hulls (%) | | 0 | 1434 | 2055$^{a}$ | 3489$^{a}$ | 1022$^{b}$ | 1288$^{b}$ | 2311$^{a}$ | 1.402 | 1.597 | 1.511$^{a}$ |
| | | 3 | 1456 | 2104$^{b}$ | 3560$^{b}$ | 1057$^{a}$ | 1338$^{a}$ | 2396$^{b}$ | 1.379 | 1.573 | 1.486$^{b}$ |
| Pellet temperature | | 75$^0$C | 1448 | 2073 | 3521 | 1038 | 1313 | 2356 | 1.397 | 1.581 | 1.499 |
| | | 90$^0$C | 1442 | 2087 | 3529 | 1043 | 1313 | 2351 | 1.384 | 1.590 | 1.498 |
| *Source of variation (P values)* | | | | | | | | | | | |
| Canola | | | 0.088 | 0.575 | 0.139 | 0.032 | 0.517 | 0.105 | <.0001 | 0.116 | <.0001 |
| Oat hulls | | | 0.078 | 0.018 | 0.009 | 0.007 | 0.003 | 0.001 | 0.088 | 0.069 | 0.010 |
| Pellet temperature | | | 0.692 | 0.503 | 0.739 | 0.639 | 0.997 | 0.803 | 0.313 | 0.501 | 0.944 |
| Canola × oat hulls | | | 0.217 | 0.030 | 0.025 | 0.004 | 0.900 | 0.134 | 0.020 | 0.009 | 0.609 |
| Canola × temp | | | 0.301 | 0.870 | 0.717 | 0.414 | 0.914 | 0.717 | 0.955 | 1.000 | 0.892 |
| Oat hulls × temp | | | 0.863 | 0.482 | 0.532 | 0.403 | 0.163 | 0.148 | 0.392 | 0.204 | 0.120 |
| Canola × oat hulls × temp | | | 0.792 | 0.075 | 0.206 | 0.101 | 0.928 | 0.340 | 0.089 | 0.057 | 0.924 |

[1]Each value for represents the mean of 6 replicates for treatment effects and 24 replicates for main effects

[2]Means within a column not sharing a superscript differ significantly at the P<0.05 level for the treatment effects and at the P level shown for the main effects.

Table 11 Relative weight organs (g/100g body weight) of broiler chickens on d 24 and 35 [1,2]

| Treatments | | | Proventriculus | | Gizzard | | Liver | | Duodenum | | Jejunum | | Ileum | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oat hulls (%) | Temp [3] | d 24 | d 35 | d 24 | d 35 | d 24 | d 35 | d 24 | d 35 | d 24 | d 35 | d 24 | d 35 |
| Canola meal | 0 | 75$^0$C | 0.46 | 0.35 | 1.87 | 1.24 | 3.10 | 2.33 | 1.08 | 0.66 | 1.79 | 1.33 | 1.20 | 0.89 |
| Canola meal | 0 | 90$^0$C | 0.50 | 0.31 | 2.17 | 1.34 | 3.00 | 2.29 | 1.14 | 0.70 | 1.99 | 1.23 | 1.25 | 0.83 |
| Canola meal | 3 | 75$^0$C | 0.49 | 0.35 | 2.19 | 1.54 | 3.06 | 2.27 | 1.00 | 0.67 | 1.97 | 1.30 | 1.19 | 0.96 |
| Canola meal | 3 | 90$^0$C | 0.49 | 0.34 | 2.29 | 1.51 | 3.00 | 2.36 | 0.99 | 0.73 | 1.82 | 1.24 | 1.33 | 0.84 |
| Canola seed | 0 | 75$^0$C | 0.46 | 0.32 | 1.93 | 1.35 | 2.93 | 2.40 | 1.02 | 0.68 | 1.92 | 1.32 | 1.23 | 0.83 |
| Canola seed | 0 | 90$^0$C | 0.49 | 0.33 | 2.12 | 1.41 | 3.11 | 2.38 | 1.18 | 0.71 | 2.07 | 1.16 | 1.31 | 0.85 |
| Canola seed | 3 | 75$^0$C | 0.47 | 0.33 | 2.28 | 1.45 | 2.97 | 2.31 | 1.06 | 0.66 | 1.94 | 1.31 | 1.19 | 0.88 |
| Canola seed | 3 | 90$^0$C | 0.48 | 0.34 | 2.22 | 1.55 | 3.00 | 2.38 | 1.13 | 0.75 | 2.12 | 1.43 | 1.21 | 0.93 |
| | | SEM | 0.006 | 0.004 | 0.023 | 0.021 | 0.028 | 0.024 | 0.016 | 0.010 | 0.023 | 0.019 | 0.020 | 0.011 |
| *Main effects* | | | | | | | | | | | | | | |
| Canola | | Meal | 0.48 | 0.33 | 2.13 | 1.41 | 3.03 | 2.31 | 1.05 | 0.69 | 1.89$^b$ | 1.27 | 1.24 | 0.88 |
| | | Seed | 0.47 | 0.33 | 2.14 | 1.44 | 3.00 | 2.37 | 1.09 | 0.70 | 2.01$^a$ | 1.30 | 1.23 | 0.87 |
| Oat hulls (%) | | 0 | 0.47 | 0.32 | 2.02$^b$ | 1.33$^b$ | 3.03 | 2.35 | 1.10 | 0.68 | 1.94 | 1.26 | 1.25 | 0.85$^a$ |
| | | 3 | 0.48 | 0.34 | 2.27$^a$ | 1.51$^a$ | 3.01 | 2.33 | 1.05 | 0.70 | 1.96 | 1.32 | 1.23 | 0.90$^b$ |
| Pellet temperature | | 75$^0$C | 0.47 | 0.34 | 2.07$^b$ | 1.40 | 3.02 | 2.32 | 1.04$^b$ | 0.67$^b$ | 1.90 | 1.31 | 1.20 | 0.88 |
| | | 90$^0$C | 0.49 | 0.33 | 2.20$^a$ | 1.45 | 3.02 | 2.35 | 1.11$^a$ | 0.72$^a$ | 1.99 | 1.26 | 1.27 | 0.86 |
| *Source of variation* | | | | | | | | | | | | | | |
| Canola | | | 0.340 | 0.691 | 0.855 | 0.448 | 0.508 | 0.256 | 0.182 | 0.529 | 0.014 | 0.423 | 0.874 | 0.704 |
| Oat hulls | | | 0.749 | 0.096 | <.001 | 0.001 | 0.657 | 0.641 | 0.055 | 0.450 | 0.683 | 0.107 | 0.619 | 0.025 |
| Pellet temperature | | | 0.253 | 0.487 | 0.006 | 0.193 | 0.832 | 0.606 | 0.024 | 0.006 | 0.056 | 0.188 | 0.067 | 0.227 |
| Canola × oat hulls | | | 0.655 | 0.842 | 0.971 | 0.186 | 0.890 | 0.629 | 0.077 | 0.737 | 0.790 | 0.060 | 0.170 | 0.621 |
| Canola × temp | | | 1.000 | 0.079 | 0.154 | 0.601 | 0.116 | 0.953 | 0.151 | 0.801 | 0.123 | 0.449 | 0.561 | 0.006 |
| Oat hulls × temp | | | 0.253 | 0.277 | 0.018 | 0.614 | 0.667 | 0.278 | 0.209 | 0.336 | 0.100 | 0.052 | 0.891 | 0.790 |
| Canola × oat hulls × temp | | | 0.701 | 0.322 | 0.784 | 0.302 | 0.395 | 0.806 | 0.779 | 0.529 | 0.044 | 0.111 | 0.338 | 0.471 |

[1]Each value for represents the mean of 6 replicates for treatment effects and 24 replicates for main effects

[2]Means within a column not sharing a superscript differ significantly at the $P<0.05$ level for the treatment effects and at the $P$ level shown for the main effects

[3]Temperature

Increasing pellet temperature from 75 to 90°C caused a reduction in ileal N digestibility at d 35 ($P < 0.05$).

Inclusion of CS in the diets depressed fat digestibility at d 24 ($P < 0.001$). At d 35, there was a significant interaction ($P < 0.05$) between CS and pellet temperature where birds fed CS diets pelleted at 75°C had higher fat digestibility than birds fed CS pelleted at 90°C. Regardless of canola or pellet temperature, OH increased fat utilization at d 35 ($P < 0.001$).

***Ileal amino acid digestibility***

Table 13 and 14 show the results for apparent ileal digestibility coefficients of indispensable and dispensable AA, respectively. There was no three-way interaction for AA digestibility. Significant interaction was found between CS and OH showing a positive effect of OH on Arg ($P < 0.05$) and Met ($P < 0.001$) digestibility in CS fed birds. Pellet temperature and CS also interacted ($P < 0.05$) for Arg, His, Iso, Lys, Met, Thr, Asp, Ser and Tyr where CS inclusion resulted in lower digestibility only when diets were pelleted at 75°C.

Inclusion of CS in the diets independently reduced ($P < 0.05$) Leu, Ala, Glu, and Pro. Addition of OH also increased digestibility of Iso ($P < 0.05$), Leu ($P < 0.05$), Phe ($P < 0.05$), Thr ($P < 0.05$), Val ($P < 0.05$), Ala ($P < 0.05$), Asp ($P < 0.01$), Gly ($P < 0.01$) and Tyr ($P < 0.05$).

***Apparent metabolisable energy of grower diets and ileal viscosity***

As shown in Table 12, inclusion of CS in the diets decreased the AME of grower diets which was observed only when a high pellet temperature was applied. Ileal viscosity was not affected by experimental treatments when assessed at d 35 of age (Table 5).

Table 12 Apparent ileal nutrient digestibility coefficients of grower (d 24) and finisher diets (d 35) and viscosity of ileal content on d 35

| | Oat hulls (%) | Temperature | DM | DM | N | N | Fat | Fat | AME | Viscosity |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | d 24 | d 35 | d 24 | d 35 | d 24 | d 35 | | d 35 |
| Canola meal | 0 | 75$^0$C | 0.718 | 0.746 | 0.821 | 0.836 | 0.833 | 0.850 | 13.06a | 1.68 |
| Canola meal | 0 | 90$^0$C | 0.705 | 0.730 | 0.819 | 0.831 | 0.833 | 0.840 | 13.06a | 1.74 |
| Canola meal | 3 | 75$^0$C | 0.718 | 0.732 | 0.830 | 0.844 | 0.816 | 0.873 | 13.05a | 1.59 |
| Canola meal | 3 | 90$^0$C | 0.707 | 0.719 | 0.822 | 0.835 | 0.834 | 0.864 | 13.01a | 1.62 |
| Canola seed | 0 | 75$^0$C | 0.673 | 0.739 | 0.805 | 0.838 | 0.768 | 0.822 | 13.03a | 1.75 |
| Canola seed | 0 | 90$^0$C | 0.704 | 0.713 | 0.809 | 0.814 | 0.754 | 0.753 | 12.64b | 1.65 |
| Canola seed | 3 | 75$^0$C | 0.689 | 0.716 | 0.812 | 0.841 | 0.789 | 0.822 | 12.72b | 1.72 |
| Canola seed | 3 | 90$^0$C | 0.703 | 0.719 | 0.831 | 0.837 | 0.764 | 0.796 | 12.76b | 1.71 |
| | | SEM | 0.0030 | 0.0025 | 0.0022 | 0.0021 | 0.0073 | 0.0036 | 0.020 | 0.033 |
| *Main effects* | | | | | | | | | | |
| Canola | | Meal | 0.712$^a$ | 0.732$^a$ | 0.823 | 0.836 | 0.829$^a$ | 0.857$^a$ | 13.04$^a$ | 1.658 |
| | | Seed | 0.692$^b$ | 0.721$^b$ | 0.814 | 0.832 | 0.769$^b$ | 0.798$^b$ | 12.78$^b$ | 1.708 |
| Oat hulls (%) | | 0 | 0.700 | 0.731$^a$ | 0.814$^a$ | 0.829$^a$ | 0.797 | 0.816$^a$ | 12.93 | 1.703 |
| | | 3 | 0.704 | 0.721$^b$ | 0.824$^b$ | 0.839$^b$ | 0.801 | 0.839$^b$ | 12.90 | 1.663 |
| Pellet temperature | | 75$^0$C | 0.700 | 0.733$^a$ | 0.817 | 0.840$^a$ | 0.801 | 0.842$^a$ | 12.96$^a$ | 1.686 |
| | | 90$^0$C | 0.705 | 0.720$^b$ | 0.820 | 0.829$^b$ | 0.796 | 0.813$^b$ | 12.86$^b$ | 1.680 |
| *Source of variation (P values)* | | | | | | | | | | |
| Canola | | | 0.002 | 0.049 | 0.067 | 0.328 | <0.001 | <0.001 | <0.001 | 0.453 |
| Oat hulls | | | 0.473 | 0.039 | 0.024 | 0.028 | 0.812 | 0.003 | 0.406 | 0.548 |
| Pellet temperature | | | 0.401 | 0.013 | 0.466 | 0.015 | 0.730 | 0.001 | 0.022 | 0.910 |
| Canola × oat hulls | | | 0.589 | 0.679 | 0.361 | 0.422 | 0.434 | 0.899 | 0.148 | 0.375 |
| Canola × temp | | | 0.007 | 0.692 | 0.078 | 0.433 | 0.323 | 0.013 | 0.069 | 0.453 |
| Oat hulls × temp | | | 0.533 | 0.120 | 0.600 | 0.358 | 0.910 | 0.137 | 0.006 | 0.802 |
| Canola × oat hulls × temp | | | 0.440 | 0.184 | 0.273 | 0.192 | 0.623 | 0.158 | 0.023 | 0.661 |

[1]Each value for represents the mean of 6 replicates for treatment effects and 24 replicates for main effects

[2]Means within a column not sharing a superscript differ significantly at the P<0.05 level for the treatment effects and at the P level shown for the main effects

Table 13 Apparent ileal digestibility coefficient of indispensable amino acids for broiler chickens fed grower diets (d 24)

| | Oat hulls (%) | Temperature | Arg | His | Iso | Leu | Lys | Met | Phe | Thr | Val |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Canola meal | 0 | 75$^0$C | 0.884 | 0.849 | 0.828 | 0.830 | 0.866 | 0.946 | 0.846 | 0.800 | 0.817 |
| Canola meal | 0 | 90$^0$C | 0.876 | 0.840 | 0.818 | 0.820 | 0.860 | 0.942 | 0.836 | 0.792 | 0.804 |
| Canola meal | 3 | 75$^0$C | 0.883 | 0.843 | 0.816 | 0.812 | 0.863 | 0.945 | 0.835 | 0.793 | 0.803 |
| Canola meal | 3 | 90$^0$C | 0.877 | 0.841 | 0.819 | 0.820 | 0.861 | 0.945 | 0.838 | 0.790 | 0.805 |
| Canola seed | 0 | 75$^0$C | 0.858 | 0.817 | 0.798 | 0.799 | 0.842 | 0.933 | 0.819 | 0.761 | 0.785 |
| Canola seed | 0 | 90$^0$C | 0.867 | 0.834 | 0.811 | 0.806 | 0.853 | 0.936 | 0.825 | 0.779 | 0.797 |
| Canola seed | 3 | 75$^0$C | 0.874 | 0.839 | 0.812 | 0.810 | 0.854 | 0.940 | 0.827 | 0.782 | 0.799 |
| Canola seed | 3 | 90$^0$C | 0.883 | 0.846 | 0.828 | 0.825 | 0.869 | 0.947 | 0.842 | 0.797 | 0.814 |
| | | SEM | 0.0016 | 0.0020 | 0.0023 | 0.0024 | 0.0020 | 0.0009 | 0.0021 | 0.0027 | 0.0024 |
| *Main effects* | | | | | | | | | | | |
| Canola | | Meal | 0.880a | 0.843a | 0.820 | 0.820a | 0.862 | 0.944a | 0.839a | 0.794a | 0.807 |
| | | Seed | 0.870b | 0.834b | 0.812 | 0.809b | 0.854 | 0.939b | 0.828b | 0.780b | 0.799 |
| Oat hulls (%) | | 0 | 0.871 | 0.833 | 0.811 | 0.809 | 0.854 | 0.940 | 0.829 | 0.780 | 0.797 |
| | | 3 | 0.879 | 0.843 | 0.821 | 0.821 | 0.862 | 0.944 | 0.838 | 0.793 | 0.808 |
| Pellet temperature | | 75$^0$C | 0.874 | 0.837 | 0.813 | 0.812 | 0.856 | 0.941 | 0.832 | 0.784 | 0.801 |
| | | 90$^0$C | 0.876 | 0.840 | 0.819 | 0.817 | 0.861 | 0.943 | 0.835 | 0.789 | 0.805 |
| *Source of variation (P values)* | | | | | | | | | | | |
| Canola | | | 0.008 | 0.025 | 0.084 | 0.035 | 0.057 | 0.006 | 0.017 | 0.012 | 0.093 |
| Oat hulls | | | 0.017 | 0.017 | 0.029 | 0.018 | 0.083 | 0.032 | 0.049 | 0.027 | 0.029 |
| Pellet temperature | | | 0.744 | 0.445 | 0.231 | 0.319 | 0.283 | 0.339 | 0.431 | 0.352 | 0.390 |
| Canola × oat hulls | | | 0.019 | 0.071 | 0.290 | 0.489 | 0.132 | 0.009 | 0.346 | 0.181 | 0.345 |
| Canola × temp | | | 0.020 | 0.036 | 0.058 | 0.218 | 0.042 | 0.047 | 0.103 | 0.049 | 0.059 |
| Oat hulls × temp | | | 0.900 | 0.285 | 0.607 | 0.601 | 0.992 | 0.911 | 0.813 | 0.728 | 0.543 |
| Canola × oat hulls × temp | | | 0.880 | 0.852 | 0.396 | 0.167 | 0.552 | 0.296 | 0.225 | 0.932 | 0.354 |

[1]Each value for represents the mean of 6 replicates for treatment effects and 24 replicates for main effects

[2]Means within a column not sharing a superscript differ significantly at the P<0.05 level for the treatment effects and at the P level shown for the main effects

Table 14 Apparent ileal digestibility coefficient of dispensable amino acids for broiler chickens fed grower diets (d 24)

| | Oat hulls (%) | Temperature | Ala | Asp | Glu | Gly | Pro | Ser | Tyr |
|---|---|---|---|---|---|---|---|---|---|
| Canola meal | 0 | 75$^0$C | 0.807 | 0.808 | 0.887 | 0.799 | 0.843 | 0.810 | 0.814 |
| Canola meal | 0 | 90$^0$C | 0.796 | 0.796 | 0.878 | 0.793 | 0.834 | 0.800 | 0.810 |
| Canola meal | 3 | 75$^0$C | 0.783 | 0.794 | 0.878 | 0.792 | 0.835 | 0.800 | 0.818 |
| Canola meal | 3 | 90$^0$C | 0.797 | 0.790 | 0.882 | 0.789 | 0.837 | 0.799 | 0.804 |
| Canola seed | 0 | 75$^0$C | 0.763 | 0.762 | 0.869 | 0.763 | 0.821 | 0.776 | 0.780 |
| Canola seed | 0 | 90$^0$C | 0.775 | 0.785 | 0.875 | 0.782 | 0.829 | 0.794 | 0.784 |
| Canola seed | 3 | 75$^0$C | 0.781 | 0.789 | 0.869 | 0.793 | 0.826 | 0.793 | 0.796 |
| Canola seed | 3 | 90$^0$C | 0.799 | 0.804 | 0.880 | 0.804 | 0.836 | 0.806 | 0.817 |
| | | SEM | 0.0031 | 0.0025 | 0.0017 | 0.0025 | 0.0020 | 0.0025 | 0.0025 |
| *Main effects* | | | | | | | | | |
| Canola | | Meal | 0.796a | 0.797a | 0.881a | 0.793 | 0.837a | 0.802 | 0.811a |
| | | Seed | 0.780b | 0.784b | 0.873b | 0.785 | 0.828b | 0.791 | 0.794b |
| Oat hulls (%) | | 0 | 0.779 | 0.782 | 0.875 | 0.781 | 0.830 | 0.792 | 0.796 |
| | | 3 | 0.796 | 0.799 | 0.878 | 0.797 | 0.834 | 0.801 | 0.809 |
| Pellet temperature | | 75$^0$C | 0.783 | 0.788 | 0.876 | 0.789 | 0.831 | 0.794 | 0.802 |
| | | 90$^0$C | 0.792 | 0.793 | 0.878 | 0.792 | 0.834 | 0.799 | 0.804 |
| *Source of variation (P values)* | | | | | | | | | |
| Canola | | | 0.013 | 0.020 | 0.028 | 0.122 | 0.025 | 0.051 | 0.001 |
| Oat hulls | | | 0.012 | 0.003 | 0.441 | 0.004 | 0.284 | 0.063 | 0.014 |
| Pellet temperature | | | 0.189 | 0.278 | 0.385 | 0.320 | 0.480 | 0.301 | 0.675 |
| Canola × oat hulls | | | 0.455 | 0.197 | 0.901 | 0.050 | 0.704 | 0.382 | 0.022 |
| Canola × temp | | | 0.262 | 0.011 | 0.101 | 0.064 | 0.131 | 0.047 | 0.030 |
| Oat hulls × temp | | | 0.479 | 0.407 | 0.567 | 0.575 | 0.559 | 0.506 | 0.190 |
| Canola × oat hulls × temp | | | 0.246 | 0.961 | 0.191 | 0.852 | 0.467 | 0.871 | 0.700 |

[1]Each value for represents the mean of 6 replicates for treatment effects and 24 replicates for main effects

[2]Means within a column not sharing a superscript differ significantly at the P<0.05 level for the treatment effects and at the P level shown for the main

## DISCUSSION

### *Growth performance of birds*

The findings of this study and that of Barekatain, et al. (2015) clearly highlights that a lower feed consumption is the main reason for the differences observed between the diets containing CS and CM. Mechanism behind lower FI of bird fed CS is not fully clear. However, possible residual isothiocyanate levels resulting from breakdown of glucosinolates may have contributed to the palatability of the diets containing CS (Tripathi and Mishra, 2007). It is known that the enzyme myrosinase is responsible for breaking glucosinolates into extremely bitter compounds of isothiocyanates and goitrin (Tripathi and Mishra, 2007) that may adversely affect feed consumption. Another observation worth mentioning was the pellet durability index of the experimental diets which was observed to be higher in the diets containing CS without OH. Therefore, hardness of the pellets may have also contributed to the differences observed for FI. Nevertheless, the BW of birds in the current study was comparable to control birds which shows that CS can replace a substantial proportion of supplemental oil without adverse effect on feed efficiency.

In the present study, neither independent nor combined effect of pellet temperature was significant for the bird performance. In the literature, there is evidence that increasing temperature above 60°C can reduce the performance of birds fed wheat based diets (Abdollahi, et al., 2013a; Bedford, et al., 2003). Abdollahi, et al. (2010) found that bird fed diets pelleted at 90°C gained more weight compared with 75°C when fed maize-based diet but there was no effect when assessed for wheat-based diets. The experimental diets in the present study comprised combination of sorghum, wheat and soybean meal as major ingredients that may have contributed to difference in observations between the studies. This study, to our knowledge, is the first that has investigated the effect of pellet temperature specifically for CS in which we found no clear effect of pellet temperature for bird performance. Shen, et al. (1983) postulated that heating *per se* may be an influencing factor in steam pelleting of CS but there was no direct comparison for the temperature in that experiment. As heat treatment is able to reduce the activity of myrosinase (Fenwick, et al., 1986), it was thought that a higher pellet temperature may possibly affect FI of birds through deactivation of myrosinase. Unfortunately, under the condition of this

experiment, it was not feasible to measure the activity of myrosinase as this enzyme acts rapidly after the seed breaks during the process of feed manufacturing. Therefore, the measurement of myrosinase on manufactured feed had little relevance. A separate *in vitro* investigation may possibly provide an answer to this question.

Independent and positive effect of OH on FCR of birds when assessed for the entire experiment is line with experiments conducted by other researchers (Jiménez-Moreno, et al., 2016; Jiménez-Moreno, et al., 2013). Jimenez-Moreno et al. (2016) showed that regardless of the feed form, inclusion of moderate amount of fibre can still enhance growth performance of broiler chickens. On other side, some recent research showed that in pelleted diet, addition insoluble fibre had limited effect (van der Hoeven-Hangoor, et al., 2014). Insoluble fibre is shown to consistently increase gizzard weight by large inclusion of hulls in broiler diets (Sacranie, et al., 2012; Svihus, et al., 2010), an observation that was also confirmed in the current trial although to a lesser extend possibly due to lower inclusion of OH. The coarse material in the diets are required to be ground to a certain critical size for that gizzard adopts itself to develop in response to the gizzard content (Sacranie, et al., 2012).

In the present study, for the first time, the effect of an insoluble source of fibre for utilization of CS we observed. There was a clear interaction between OH and the type canola included in the diets for grower phase of feeding. When diets contained CS without OH, a lower FI was observed compared with other group of birds which concurs with a previous study (Barekatain, et al., 2015). However, OH offered no synergistic effect on FI but rather was effective in maintaining the FI of birds fed CS compared to control birds. Although the exact mechanism behind this interaction cannot be immediately explained by the measured nutrient utilization, the differences between fibre content of the diets and possible effect of pellet hardness may be among the contributing factors.

***Nutrient utilization***

Inclusion of CS in the diets resulted in a distinct retardation of fat utilization both in grower and finisher phase of feeding. This reduction was exacerbated by higher temperature only in finisher diets most likely due to higher inclusion of CS compared with grower diets. It is well documented that some of the oil in CS remains

encapsulated in the peptide shell oil bodies that in turn impede maximum fat utilization (Barekatain, et al., 2015; Slominski, et al., 2006). The fat utilization of CS appears to be, at least to some extent, dependent on pelleting condition such as steam pelleting (Barekatain, et al., 2015) and in this experiment pellet temperature that was, however, not reflected into any performance difference for the birds. The experiments conducted for the effect pellet temperatures on fat utilization are very limited. While Jimenez-Moreno, et al. (2009) showed that steam cooking may increase fat utilization in maize based diet, Abdollahi, et al. (2013b) found that ileal fat digestibility varied depending on cereal composition of the diets. While it is unknown why a higher temperature has impaired the fat utilization only in the diets fed CS, one plausible explanation may be the difference in fatty acid composition between control and CS diet. Nevertheless, we previously found that applying steam to the diets reduced fat digestibility in birds fed CS compared with cold-pellet diets (Barekatain, et al., 2015).

Inclusion of OH improved fat digestibility in finisher diets although there was no interaction with CS inclusion. This result concurs with other studies in the literature and has been attributed to the possible beneficial effect of dietary fibre on gizzard development and subsequent increase in bile acids and digestive enzyme section. Indeed, the gizzard development was evident in the current study as a result of OH inclusion. A well functioned gizzard offers a wide range of benefit including increase antiperistaltic movement (Sacranie, et al., 2012), better mixing of digestive juices with digesta as well as reduction in pH of the hindgut which consequently may benefit enzyme activation (Mateos, et al., 2012). All these may provide explanation for observed improvement in nutrient digestibility resulted from OH.

In the present study, it was hypothesized that inclusion OH may improve fat digestion of the diets containing CS. The results showed that the effect of OH for improved fat digestibility in finisher diets was independent of CS inclusion. Nevertheless, Jiménez-Moreno et al. observed an improvement in fat digestibility when 3% OH was included in the diet of broilers in a 21-d study. Similar benefits for fat utilization resulted from inclusion of an insoluble fibre source in broiler diets have been reported (Gonzalez-Alvarado, et al., 2007; Kalmendal, et al., 2011).

There was no effect of OH inclusion on AME of grower diets which could be related to the fact that all the diets were formulated to be isoenergetic. In an experiment conducted by Sacranie et al. (2012), diets were simply diluted with OH resulted in a

substantial reduction of AME which could then be improved by a higher nutrient utilization in the birds. Given the lack of effect of OH in birds fed the control diets, it appears that balancing the diets for energy and nutrient when formulating diets is of particularly importance in response to OH inclusion. Leeson et al. (1996) showed that dilution of diets with 7.5% OH caused an increase in FI as a sign of ability of the birds to regulate their FI in response to dietary energy. Therefore, in agreement with several other studies (González-Alvarado, et al., 2010; Jiménez-Moreno, et al., 2016; Mateos, et al., 2012), the response to dietary fibre in terms of growth performance may vary depending on several factors including but not limited to the composition of basal diets, age, health status, genetic potential (Jiménez-Moreno, et al., 2016).

There were lower AA digestibility coefficients for 11 AA in the diet containing CS compared with canola meal in control diets. This observation in in disagreement with a previous study in which the CS used contained higher amount of oil and the diet were pelleted in average at a lower temperature (Barekatain, et al., 2015).

## CONCLUSION

It can be concluded that CS can be used in poultry diets as whole in steam pelleted diets containing a moderate amount of insoluble fibre such as 3% OH. Inclusion of OH appears to be help birds to maintain the consumption of feed which otherwise would be adversely affected by inclusion of CS in the diets. It is however acknowledged that commercially, inclusion of 3% OH would be expensive but improvement resulted from that may offset the costs. Under the condition of this experiment, birds were not responsive to pellet temperature for performance parameters. However, some negative effect of high pelleting temperature on nutrient utilization, fat in particular, was apparent which may point to a suggestion to maintain the pellet temperature at around 75°C when CS is included in broiler diets.

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# Chapter 4 General conclusion

This project resulted complemented the previous project 2.1.8 supported by the Poultry CRC in which the feasibility of replacing supplemental oil by CS was investigated. The results of both projects provided unequivocal evidence that CS possess a high potential to be included in the diet of broiler chickens at higher levels than current industry practice. Inclusion of CS in the diets, however may depress the feed consumption but can be compensated by a better feed conversion as shown in the current experiment and that of Barekatain et al. (2015). There is considerable variation in the nutrient composition of canola seed from different geographical locations around Australia as shown in Chapter 3. This was even apparent from the samples obtained from a same location but over different times. As shown in Chapter 3, the AME and AMEn values of seed samples ranged from 4,728 to 5,071, and 4,501 to 4,791 kcal/kg of DM, respectively which highlight the need for accurate data for energy and nutrient when formulating diets containing CS. Correlations performed between the nutrient composition and energy utilization were found to be not very strong that was the reflection of the fact that samples showed considerable variations for fibre, fat and protein that are known to differ in their contribution to energy. Increasing number of CS samples subjected to AME assay may eventually provide a reasonable equation for nutritionist to use as an alternative to AME assay for CS.

As shown in Chapter 4, birds fed CS had similar BW compared with bird fed control diets. The results for grower phase of feeding showed that if an insoluble source of fibre such as OH is added to the diets, feed intake can be maintained similar to control birds. This was evident with a significant interaction between the OH inclusion and source of canola in the diets. Under the condition of these experiments, birds were not responsive to the 15°C difference between 75 and 90°C for the pellet temperature. However, there were marginal adverse effect on nutrient utilization in particular fat digestibility when diets were pelleted at 90°C. A wider range of pelleting temperature may possibly provide a better picture of the effect of temperature on utilization of CS in broilers.

**Plain English Compendium Summary**

| | |
|---|---|
| **Sub-Project Title:** | Interactions of canola seed source, pellet temperature and fibre for broilers |
| Poultry CRC Sub-Project No.: | 2.1.10 |
| Researcher: | M.R. Barekatain |
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| **Sub-Project Overview** | Increase productivity and efficiency of broilers fed canola seed |
| **Background** | The use of canola seed in poultry diets has become common following an increase in price of fat and oil for use in broiler feed. Subsequently, broiler diets have increased in cost. Some Australian broiler producers are including full fat canola seed (CS) in broiler diets. Canola seed can contribute substantially more to the metabolisable energy content of the diet than oil-extracted solvent or expeller canola meal. In this regard, little information is available for the variation and nutritive value of CS grown in Australia. The current subproject was proposed based on the research gaps outlined in previous CRC sub-project 2.1.8; in particular, there is a need to further examine the variation between canola seed sources and the effect of pellet temperature and dietary insoluble fibre on utilisation of nutrient of CS for broilers. In addition, the effect of fibre and pellet temperature on fat digestibility and apparent metabolisable energy of the diets were investigated. |
| **Research** | The present study demonstrates that chemical composition of canola seed has a significant effect on its energy availability for broiler chickens. Therefore, nutritionists should be cautious of the source of data for AMEn values of CS when formulating diets to reduce feed costs and also improve bird performance. Increasing conditioning temperature during steam pelleting up to 90ºC was not effective in releasing more energy from CS, but improved pellet durability index of the diets. CS can be used in poultry diets as whole in steam pelleted diet containing a moderate amount of insoluble fibre such as 3% OH. Inclusion of oat hulls (OH) appears to be help birds to maintain the consumption of feed which otherwise would be adversely affected by inclusion of CS in the diets. No distinct effect was pellet temperature (75 vs 90°C) was found for performance of the birds. |
| **Implications** | Replacing supplemental oil with CS in the diet can be fully practiced provided an accurate estimate of nutrient composition of seed is made available. A moderate amount of insoluble fibre present in the diets may help bird to maintain feed intake. |
| **Publications** | Barekatain, M. R., Toghyani, M. and Swick, R. A. 2016. Addition of oat hulls in broiler diets improves utilisation of full fat canola seed. In: Proceedings of the Australian Poultry Science Symposium, University of Sydney, Australia. 27:147.<br><br>Two journal manuscripts have been prepared and are to be submitted to Poultry Science for peer-reviewed publication. |